Accepted: 8 May 2017

DOI: 10.1111/jcpe.12743

ANIMAL EXPERIMENT

WILEY Journal of Clinical Periodontology

The aggravation of arthritis by periodontitis is dependent of IL-17 receptor A activation

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Funding information
This work was supported by the Brazilian founding agencies: CAPES (process: 3758/10-9) and FAPESP (grant #2008/08142-0 and 2009/00341-7).

Abstract

Aim: To evaluate whether *Porphyromonas gingivalis*-induced periodontitis aggravates the antigen-induced arthritis (AIA) model, and whether this effect is dependent on the Th17/IL-17 signalling pathway.

Materials and methods: Antigen-induced arthritis was triggered by local injection of methylated bovine serum albumin into the knee joint of previously immunized C57BL/6 wild-type (WT) and IL-17 receptor A (IL-17RA)-knockout mice. Periodontal disease in naïve or arthritic mice was induced by oral infection with *P. gingivalis*. Animals were sacrificed 7, 15 and 30 days after infection. Alveolar bone loss, joint histopathology, articular hyperalgesia and joint cytokine production were assessed, in addition to the proportion of Th17 and Treg cells isolated from the inguinal lymph nodes.

Results: No influence of experimentally-induced arthritis was found on the alveolar bone resorption induced by *P. gingivalis*. However, mice with experimentally-induced arthritis that were exposed to *P. gingivalis* presented higher joint damage and Th17 frequencies when compared to non-infected mice. The aggravation of arthritis by periodontitis was accompanied by increased TNF and IL-17 production and articular neutrophil infiltration, whereas arthritis aggravation and changes in neutrophil infiltration were absent in IL-17RA-deficient mice.

Conclusion: The effects of *P. gingivalis*-induced periodontitis on arthritis are dependent on Th17 expansion and IL-17RA signalling, which lead to increased neutrophil infiltration into the joints.

KEYWORDS

antigen-induced arthritis, IL-17, oral microbiota, periodontal disease, *Porphyromonas gingivalis*, rheumatoid arthritis

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterised by chronic inflammation of the joints, with subsequent cartilage and bone destruction. Articular damage involves massive leukocyte recruitment (neutrophils), which is mediated by cytokines including tumour necrosis factor (TNF) and interleukin (IL)-17 (Brennan & McInnes, 2008). The T helper (Th)-17 cell subset plays an important role in articular inflammation during the early stage of RA, controlling the recruitment and activation of other inflammatory cells leading to increased production of IL-17 (Miossec & Kolls, 2012). Indeed, IL-17 receptor A (IL-17RA) signalling mediates the production of chemokines,

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proinflammatory cytokines and matrix metalloproteinases (MMPs), which are secreted into the joint space. Additionally, Th17/IL-17 activity plays a crucial role in osteoclastogenesis and bone resorption (van Hamburg et al., 2011; Miossec & Kolls, 2012). Moreover, it has been shown that the synergistic activity of TNF and IL-17 aggravates articular damage via the upregulated expression of proinflammatory cytokines and MMPs (Koenders et al., 2011).

Even though the etiology of RA remains unknown, epidemiological studies have reported an association between the development and progression of RA with exposure to environmental factors such as smoke and infections, like periodontitis (Leech & Bartold, 2015). Periodontitis is defined as the loss of attachment and bone destruction around the teeth, induced by a chronic immune inflammatory response to an infection to the periodontal biofilm. *Porphyromonas gingivalis* is the major periodontal pathogen involved in the development and progression of chronic periodontitis (Holt, Ebersole, Felton, Brunsvold, & Kornman, 1988).

A growing body of evidence has implicated periodontal pathogens in the development and progression of RA (de Pablo, Chapple, Buckley, & Dietrich, 2009). Interestingly, increased antibody titers against *P. gingivalis* (Mikuls et al., 2009) and the DNA of oral bacterial, including *P. gingivalis*, have been detected in the serum and synovial fluid of RA patients (Martinez-Martinez et al., 2009; Moen et al., 2006; Reichert et al., 2013). Case-control studies found a more prevalent and severe periodontal disease (PD) status in RA patients (Al-Shammari, Al-Khabbaz, Al-Ansari, Neiva, & Wang, 2005; Bozkurt, Yetkin Ay, Berker, Tepe, & Akkus, 2006), as well as a reduction in the clinical parameters of active RA following effective periodontal treatment and control of periodontal infection (Al-Katma, Bissada, Bordeaux, Sue, & Askari, 2007; Bıyıkoğlu et al., 2013; Ortiz et al., 2009). On the other hand, clinical parameters of periodontal disease were improved in RA patients under treatment with anti-B lymphocyte therapy (Coat et al., 2015). However, it is remains unclear whether there is an etiological link between these two chronic inflammatory diseases, or whether the epidemiological results are due to statistical bias reinforced by the common risk factors (Farquharson, Butcher, & Culshaw, 2012). There are only few animal models available for preclinical investigations into this epidemiological link, and restrictions in clinical trial protocols represent major limitations for studies. Moreover, the potential association between these two diseases appears to be dependent on the experimental conditions used in the study, such as the preclinical model and bacteria investigated. Animal studies in different experimental models have demonstrated that *P. gingivalis* influences arthritis onset and severity at both the macroscopic and histological levels (Bartold, Marino, Cantley, & Haynes, 2010). Moreover, chronic inflammatory lesions induced by subcutaneous injection of heat-killed P. *gingivalis* were found to accelerate the development of adjuvant arthritis in rats (Bartold et al., 2010). Similarly, *P. gingivalis*-induced periodontitis exacerbated the severity of collagen antibody-induced arthritis (Cantley, Haynes, Marino, & Bartold, 2011; Marchesan et al., 2013). However, the mechanism by which *P. gingivalis*-induced periodontitis modulates RA progression is not fully understood, and this warrants further mechanistic studies. *Porphyromonas gingivalis* produces virulence factors that could modulate the host's adaptive immune response, which may contribute to the development of periodontitis and possibly RA aggravation (Darveau et al., 2004; Monteiro et al., 2009). Indeed, we have recently demonstrated that *P. gingivalis*-induced periodontitis aggravates collagen-induced arthritis (CIA) through modulation of the CD4+ T cell phenotype to favour Th17 balance. This effect was dependent on the presence of a pathogen-associated molecular pattern receptor, Toll-like receptor-2 (TLR2) (de Aquino et al., 2014). Here, we evaluated whether *P. gingivalis*-induced periodontitis aggravates an antigen-induced arthritis (AIA) model. We further investigated whether the Th17/IL-17 signalling pathway represents a potential mechanism underlying AIA aggravation by periodontitis, serving as a general mechanism for arthritis aggravation by periodontitis.

Clinical Relevance

Scientific rationale for the study: A growing epidemiologic evidence has been stressed the potential association between periodontitis and rheumatoid arthritis. However, this process is still largely unknown considering the controversial findings of mechanistic animal studies as well as the limitations of clinical trials to support the current evidences.

Principal findings: IL-17 receptor A activation is involved in the increased neutrophil recruitment into the joint and aggravation of antigen-induced arthritits by *P. gingivalis* oral infection.

Practical implications: This study reinforces a Th17/IL17-dependent mechanism by which *P. gingivalis* can aggravate arthritis and highlights the importance of early periodontitis management in rheumatoid arthritis patients.

2 | METHODS

2.1 | Animals

The experiments were performed on adult male C57BL/6 WT and IL-17RA-knockout (KO) mice with a C57BL/6 genetic background, all weighting between 18–22 g. Mice were housed in the animal facility of the Department of Pharmacology, School of Medicine of Ribeirao Preto, University of São Paulo (USP), Brazil, and received water and food ad libitum. The study protocols were approved by the Ethical Committee for Animal Experimentation (CEEA) of the School of Dentistry, UNESP (process no. 21/2008), and all experiments were performed in accordance with the guidelines of the National Council for the Control of Animal Experimentation (CONCEA) and Animal Research: Reporting of In Vivo Experiments (ARRIVE).

2.2 | Experimental design

The C57BL/6 mice were randomly divided into four groups (n = 5 in each): control (C) group including non-infected naïve mice that

received oral inoculations of 2% carboxymethylcellulose (CMC); experimental arthritis (AR) group including mice submitted to AIA with methylated bovine serum albumin (mBSA); periodontal disease (PD) group consisting of mice infected with *P. gingivalis* (oral inoculations of 10^8 colony-forming units (CFU) per day for 5 days, starting 1 day prior to the final immunisation); and the ARPD group which were submitted to AIA followed by *P. gingivalis* inoculation, starting 1 day before the final immunisation. Animals were sacrificed at day 7, 15, 30 and 45 after baseline, as shown in the experimental design (Figure 1). Experiments evaluating neutrophil recruitment into the articular joint were performed up to day 7. For this, the WT and Il-17RA-KO mice were divided into two groups (n = 5), AR and ARPD. Mice were sacrificed 7 hr after an intra-articular challenge was performed with mBSA (detailed protocol below).

2.3 | Experimental induction of arthritis

Mice were immunised as previously described (Grespan et al., 2008). C57BL/6 mice were sensitised with a subcutaneous injection of 500 μg of mBSA (Sigma-Aldrich, St. Louis, MO) dissolved in an emulsion containing 0.1 ml phosphate buffered saline (PBS) and 0.1 ml of complete Freund's adjuvant (CFA; 1 mg/ml of *Mycobacterium tuberculosis*; Sigma-Aldrich). Booster injections of mBSA in incomplete Freund's adjuvant (IFA; Sigma-Aldrich) were given 7 and 14 days after the first immunisation. Non-immunised (sham) mice received similar injections without mBSA. Arthritis was induced in immunised mice 21 days after the first immunisation by intra-articular injection of mBSA into the tibiofemoral joint at 10 or 30 μg/cavity dissolved in 10 μl of saline according to the specific analysis (10 μg for analyses to measure articular hyperalgesia, joint cytokine production and the proportion of Th17 and Treg cells isolated from inguinal lymph nodes; and 30 μg for joint histopathological analysis, where a second intra-articular injection was performed 4 days after the first challenge.

2.4 | Experimental induction of periodontitis

The bacterial culture and periodontal infection protocols were performed as described previously (de Aquino et al., 2014). Experimental periodontitis was induced by oral inoculation with 1×10^8 CFU of *P. gingivalis* ATCC 33277 (anaerobically grown on supplemented blood agar) diluted in 75 μl of phosphate-buffered saline (PBS) with 2% CMC. A total of five oral inoculations were consecutively performed on alternate days, beginning 1 day prior to the last immunisation with mBSA. The control group consisted of non-infected mice that received only 2% CMC on the same schedule.

2.5 | Assessment of periodontitis

The extent of alveolar bone loss was measured in the hemisected maxillae of animals sacrificed at days 30 and 45, which had been stained with methylene blue. Immediately after sacrifice, the maxillae were collected and stored overnight in 3% hydrogen peroxide solution. The remaining soft tissue was mechanically defleshed. The palatal faces of the first and second molars were positioned perpendicular to the microscope light path and photographed at 40× magnification using a stereomicroscope (MZ6; Leica). All images were analysed by a single blinded examiner using Image J 1.34 software. The area between the cementoenamel junction (CEJ) and the alveolar bone crest (ABC) around the first and second molars, in addition to the furcation length of the first molar, were measured in arbitrary units of area (AUA).

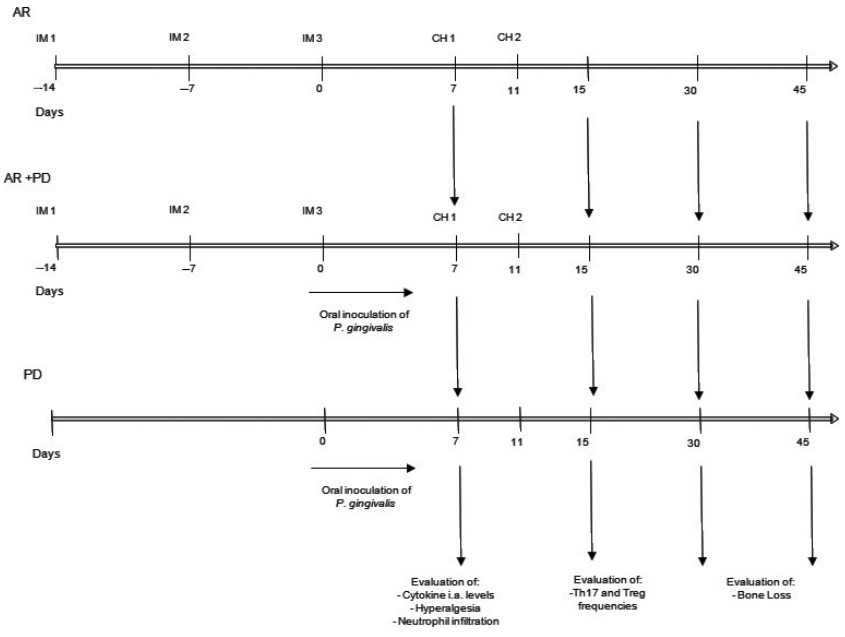

FIGURE 1 Experimental design. Schematic representation of induction of experimental arthritis (AR), *Porphyromonas gingivalis*-induced periodontitis (PD) or concomitant experimental arthritis and periodontitis (AR+PD) and timeline of immunological and histological assessment. IM: AIA immunization; CH: intraarticular antigen challenge. Five oral inoculations were consecutively performed on alternate days with *P. gingivalis*, starting at day 0

2.6 | Assessment of arthritis

2.6.1 | Histology

Knee joint inflammation was assessed histologically. Briefly, the entire knee joints of the animals sacrificed at day 30 were collected, fixed for 48 hr in 4% paraformaldehyde (PFA), then decalcified in 10% ethylenediaminetetraacetic acid (EDTA) solution (pH 7.4 in PBS) and embedded in paraffin. Serial sagittal sections of 6 μm were stained with haematoxylin and eosin (H&E), then scored by a blinded examiner on a scale from 0 to 3 according to the synovial thickness, inflammatory infiltrate and cartilage/bone erosion, as previously described (Dudler, Renggli-Zulliger, Busso, Lotz, & So, 2000; van Holten et al., 2004). Proteoglycan depletion was also scored on safranin O-stained sections using a scale from 0 (weak) to 3 (strong) based on the staining intensity.

2.6.2 | Mechanical nociceptive paw test for articular hyperalgesia

Mechanical hyperalgesia of the tibiofemoral joint was evaluated as previously reported (Pinto et al.). In a quiet room, mice were placed in acrylic cages (12 × 10 cm, 17 cm high) with a wire grid floor 15–30 min before testing. The test consisted of inducing a hindpaw flexion reflex with a handheld force transducer (electronic anesthesiometer; IITC Life Science, Woodland Hills, CA) with a large polypropylene tip (4.15 mm; IITC Life Science). The investigator applied the tip perpendicular to the central area of the plantar hindpaw, and the pressure was gradually increased to induce flexion of the tibiofemoral joint followed by paw withdrawal. The intensity of the pressure applied was automatically recorded after paw withdraw using an electronic pressure meter, and the final value for the response was obtained by taking the average of three subsequent consistent measurements. Considering that the nociceptive plateau is reached between 7 and 24 hr (based on previous data from our group), articular hyperalgesia was measured 7 hr after the challenge. Measurements obtained for the flexion-elicited mechanical threshold are expressed as grams (g).

2.6.3 | In vivo neutrophil infiltration into the joints

Neutrophil migration was assessed on day 7 after the intra-articular injection (challenge) of saline or mBSA (10 μg/cavity) in naïve or immunised mice with or without oral *P. gingivalis* infection, as described previously. Mice were sacrificed 7 hr after the challenge, and cells from the articular cavities were harvested by washing the cavity with PBS containing 1 mM EDTA. The content collected was further diluted for subsequent leucocyte migration analysis. Firstly, a total cell count was performed by diluting the samples in Turk's solution then counting the cells using a Neubauer chamber. A differential cell count was then performed by light microscopy on Rosenfeld-stained slides prepared using a cytocentrifuge. Data are expressed as the number (mean ± *SEM*) of neutrophils per cavity for each experimental group.

2.6.4 | Production of cytokines in the joints

The levels of TNF, IL-1β and IL-17 in the tibiofemoral joint induced by the mBSA challenge were quantified by enzyme-linked immunosorbent assay (ELISA). The cytokine levels were measured 7 hr after either mBSA injection (10 μg) or saline injection. Briefly, the knee joints from the injected and control sides of the animals were removed, then frozen with liquid nitrogen, triturated and homogenised in 300 μl of PBS. The homogenates were centrifuged at 10,000 *g* for 10 min, and the supernatants were collected for cytokine analyses by ELISA using paired antibodies (R&D Systems), as described previously (Pinto et al., 2010). The results are expressed as picograms per joint (pg/joint) for each cytokine.

2.6.5 | Flow cytometry analysis of T cell phenotype

After sacrifice on day 15, the inguinal lymph nodes from mice of all groups were isolated and disrupted for subsequent staining of Th17 and Treg cells. Briefly, CD4+ T cells were stained with an anti-CD4-APC antibody, washed with FACS buffer (PBS including 1% BSA), then fixed in PBS containing 2% PFA. Cells were then stained with anti-IL-17-PE and anti-forkhead box P3 (FoxP3) antibodies in FACS buffer. An appropriate isotype-matched control antibody was used for all FACS analyses. All antibodies were purchased from BD Biosciences-Pharmingen. Cells were analysed by a FACS Canto II flow cytometer using FACS Diva and CellQuest software (BD Biosciences-Pharmingen).

2.7 | Statistical analysis

Data are presented as mean ± *SEM*, and are representative of two separate experiments. The means from different groups were compared by ANOVA with Tukey or Bonferroni correction. Statistical significance was set at $p < .05$. All statistical tests were performed using GraphPad Prism version 4.0 software (GraphPad Software Inc., San Diego, CA).

3 | RESULTS

3.1 | Experimental arthritis does not modulate *Porphyromonas gingivalis*-induced alveolar bone resorption

We initially investigated whether arthritis could influence the progression of experimental PD in vivo. For this purpose, we evaluated the severity of PD induced by *P. gingivalis* in mice subjected to AIA. Hemisected maxillae stained with methylene blue were used to evaluate bone resorption at 30 (data not shown) and at 45 days after PD induction. As expected, the first and second molars of C and AR mice showed reduced alveolar bone loss than PD and ARPD mice (Figure 2a). The images and results for furcation length and the area between CEJ and ABC also suggest that the co-induction of AIA and PD did not increase alveolar bone loss compared to mice subjected to PD alone at shorter (data not shown) and longer timepoints (Figure 2b,c).

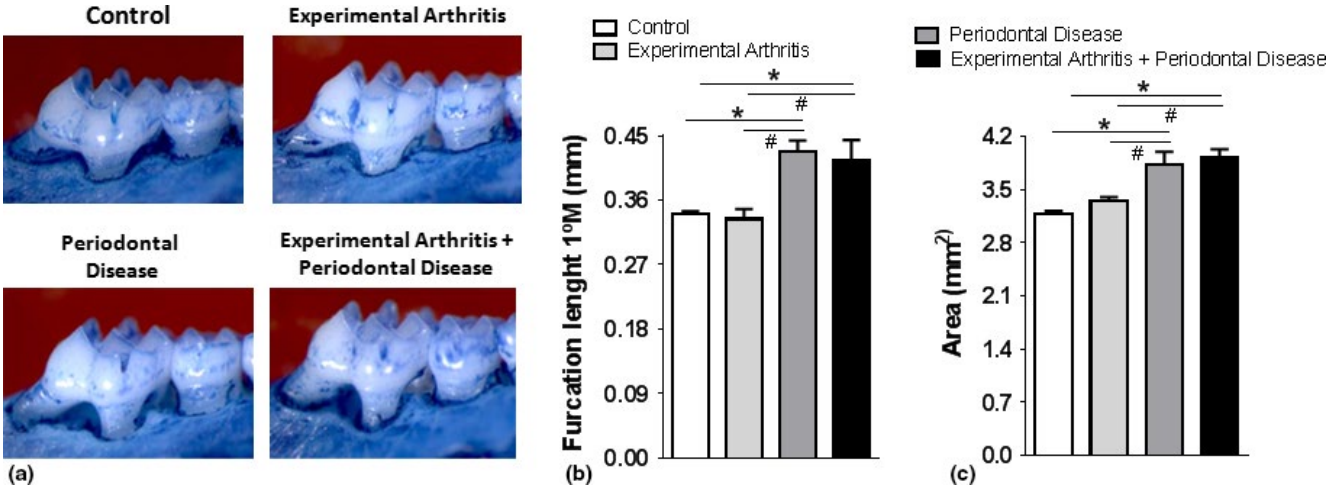

FIGURE 2 Experimental arthritis does not modulate alveolar bone loss induced by *Porphyromonas gingivalis*. C57BL/6 mice were orally infected with *P. gingivalis* as described in the methods section. (a) Representative images of hemimaxilaes evaluated for quantification of alveolar bone loss by staining with methilene blue at 45 days after infection. (b) Histomorphometrical alveolar bone loss quantification by evaluation of CEJ-ABC distance in the furcation region of 1st maxillary molar in the palatal face of maxillary molars. (c) Histomorphometrical alveolar bone loss quantification by evaluation of CEJ-ABC area around 1st and 2nd maxillary molars in the palatal face of maxillary molars. Results are represented by mean ± *SEM*. **p* < .05 as compared to control; #*p* < .05 as compared to experimental arthritis obtained by ANOVA followed by Bonferroni's test, *n* = 5

3.2 | Periodontitis induced by *Porphyromonas gingivalis* increases the severity of articular damage in an antigen-induced arthritis model

We evaluated histological changes in the joint induced by AIA in mice infected with *P. gingivalis* in order to further confirm that PD could influence the progression of experimentally-induced arthritis. Non-challenged mice (C) and PD mice did not present any evidence of synovial inflammation, bone erosion or proteoglycan loss, which were all observed in AR mice (Figure 3a–d). Importantly, the combination of PD and AR induced more severe articular damage, suggesting that *P. gingivalis* enhanced the severity of AR. Periodontal disease induced by *P. gingivalis* was associated with higher inflammatory (Figure 3a,c) and proteoglycan loss (Figure 3b,d) scores when compared to all other groups. The presence of periodontitis also increased the influx of inflammatory cells into the articular cavity, with a higher number of mononuclear cells accompanied by pannus formation (synovial hyperplasia) (Figure 3a). Thus, the histological findings further support the pathogenic role of *P. gingivalis*-induced PD on the disease severity of experimentally-induced arthritis.

3.3 | Periodontitis induced by *Porphyromonas gingivalis* increases IL-17 secretion into the inflamed joints of mice with antigen-induced arthritis

It has previously been reported that an imbalance in Th17/Treg cells plays an important role in the immunopathological coordination of arthritis inflammation (Cope, Schulze-Koops, & Aringer, 2007). In order to investigate whether the aggravation of arthritis by experimentally-induced periodontitis was mediated by changes in the T cell phenotype, analysis of the total T cells in draining lymph nodes was performed. While PD did not induce any change in the proportion of Th17 cells in the inguinal lymph nodes of naïve mice, AR mice showed a higher frequency of Th17 cells when they had been previously infected with *P. gingivalis* (Figure 4a), supporting our observation of aggravation of RA by PD. Interestingly, we did not observe any change in Treg frequencies in the draining lymph nodes as a result of periodontitis (Figure 4b). However, increased levels of the proinflammatory cytokines IL-17 and TNF were observed in the articular tissues of mice with concomitant AIA and periodontitis (Figure 4c,d).

3.4 | *Porphyromonas gingivalis*-induced periodontitis increases neutrophil infiltration into the joints of mice with antigen-induced arthritis in an IL-17RA-dependent manner

Our group has previously reported that IL-17 production in inflamed joints is related to the induction of chemokines and recruitment of neutrophils into the articular tissue (Pinto et al., 2010). Articular damage is associated with neutrophil infiltration into the articular tissue in the early stages of arthritis, therefore, we evaluated whether the aggravation of arthritis by periodontitis was related to altered neutrophil migration into the joints. Among the mice with AIA, neutrophil infiltration into the articular cavity was found to be higher in mice with concomitant periodontitis than in non-infected mice (Figure 5a). This was followed by significantly increased articular hyperalgesia in mice subjected to co-induction of arthritis and periodontitis compared to mice with arthritis only (Figure 5b). Interestingly, periodontitis did not induce increase neutrophil migration in the IL-17RA-KO mice (Figure 5c), suggesting that aggravation of arthritis by *P. gingivalis* is dependent on functional IL-17/IL-17RA signalling.

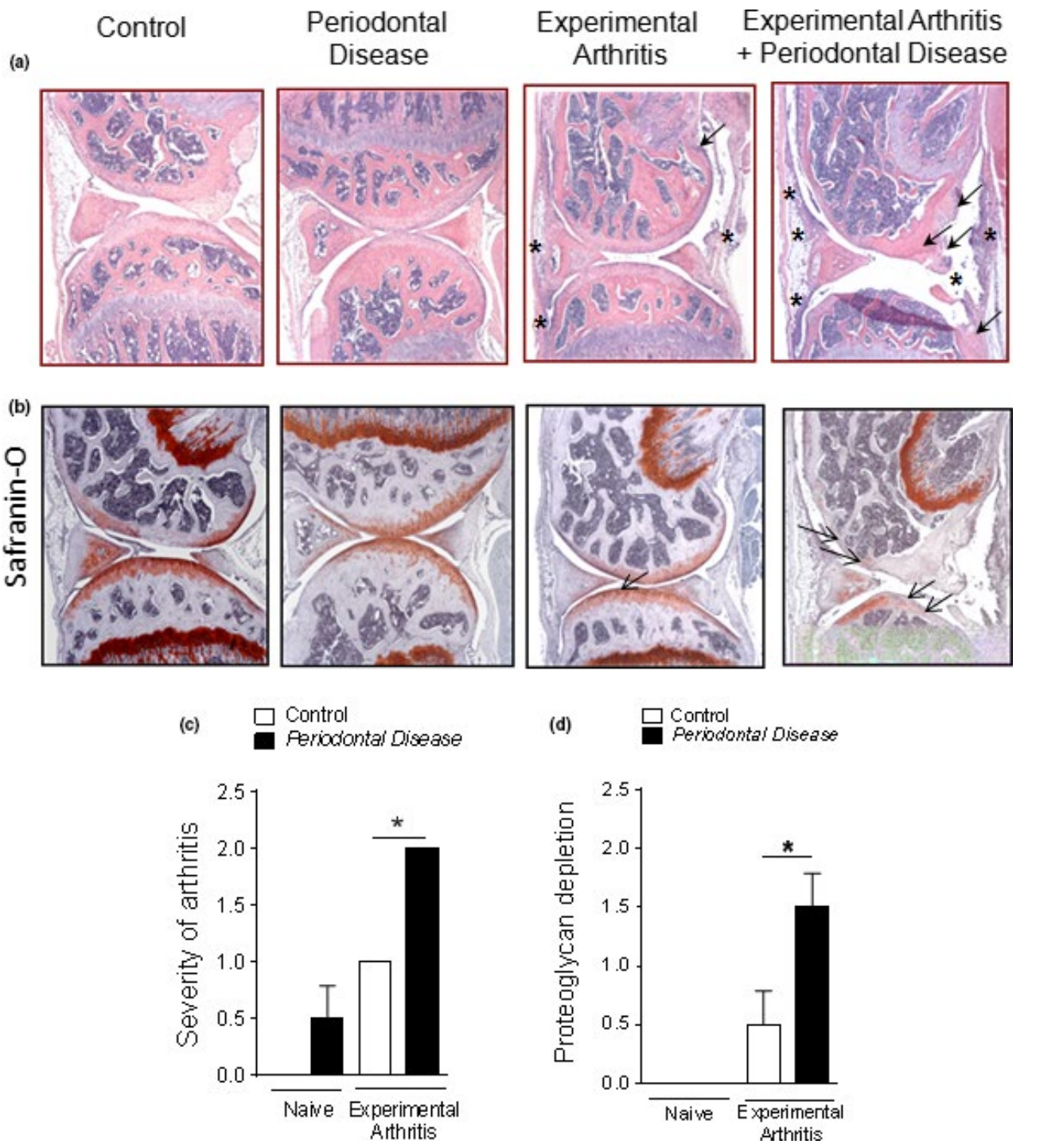

FIGURE 3 *Porphyromonas gingivalis*-induced PD enhances the severity of articular injury during experimental arthritis. (a, b) Representative histological images of femur-tibial joint of mice 30 days after *P. gingivalis* infection; upper panel shows H&E stained sections: stars and stealth arrows indicate inflammatory infiltrate and bone erosion, respectively; lower panel shows safranin O staining (red) and open arrows indicate proteoglican depletion. Representative scores of severity of inflammatory arthritis (c) and proteoglican depletion (d) – period of 30 days showing higher articular inflammatory scores and proteoglycan loss in mice subjected to co-induction of antigen-induced arthritis and *P. gingivalis* infection (ARPD group). The results are expressed as mean ± *SEM* and $^*p < .05$ as compared to control (ANOVA followed by Tukey test), $n = 5$

4 | DISCUSSION

In light of the emerging role of microbiota in autoimmune diseases, this study demonstrates that aggravation of arthritis induced by oral *P. gingivalis* infection is dependent on IL-17RA activation and subsequent increased neutrophil infiltration into the joints. However, until now, the mechanisms involved in the connection between PD and RA is still not well understood and presents controversial findings.

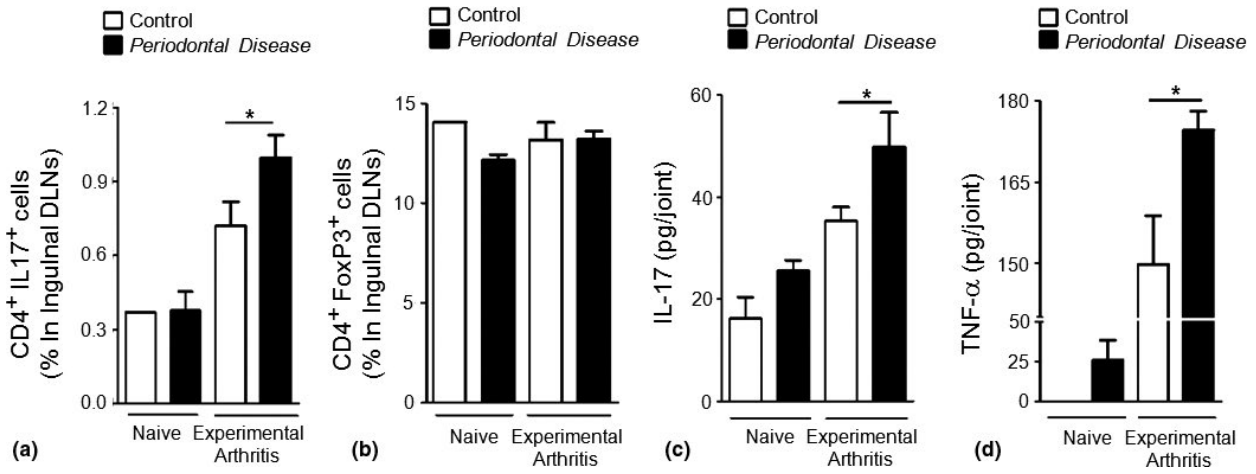

FIGURE 4 Periodontitis induces increase of Th17 frequencies and intraarticular production of IL-17 and TNF in mice with AR. (a) Flow cytometry analysis of Th17 (CD4+ IL+17 cells) frequencies in the inguinal draining lymph nodes at day 15 after infection. (b) Treg (CD4+ FOXP3+) frequencies in the inguinal draining lymph nodes at day 15 after infection. Cells were analyzed on a FACSCalibur flow cytometer using the CellQuest software (BD Biosciences-Pharmingen). (c and d) Intra-articular levels of the pro-inflammatory cytokines IL-17 (c) and TNF (d) 7 hr after antigen challenge (CH1 -10 μg/cavity) at day 7 after infection. The results are expressed as mean ± *SEM* and $^*p < .05$ as compared to control (ANOVA followed by Tukey test), $n = 5$

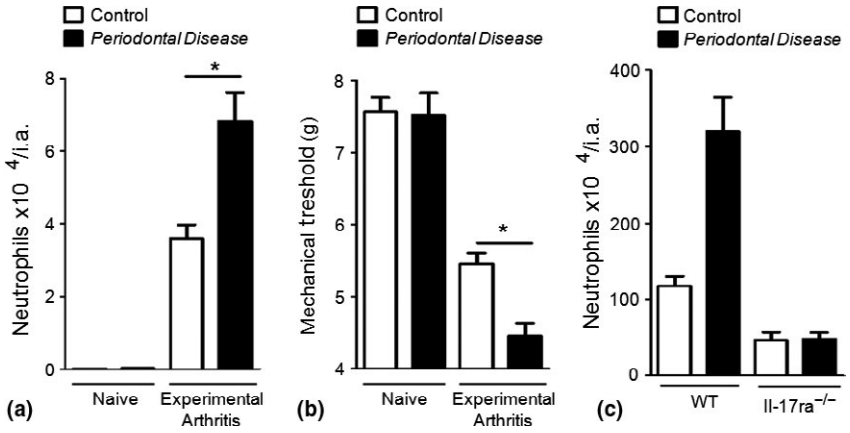

FIGURE 5 Periodontitis increase neutrophil infiltration into the joints of mice under experimental arthritis in IL-17RA-dependent manner. (a) Number of neutrophils in articular lavage 7 hr after antigen challenge (CH1 – 10 μg/cavity) (at day 7 after oral infection). (b) Mechanical articular hyperalgesia 7 hr after CH1. (c) Number of neutrophils in articular lavage 7 hr after antigen challenge (CH1) in wild-type (WT) or IL-17RA genetic deficient mice (Il-17ra-/-). (a-c). The results are expressed as mean ± *SEM* and $^*p < .05$ as compared to control (ANOVA followed by Tukey test), $n = 5$

Previous studies on the potential role of *P. gingivalis* on RA have primarily focused on its ability to citrullinate proteins and generate potential autoantigens (Hitchon et al., 2010; Wegner et al., 2010). Herein, we have confirmed, using the AIA model, that the involvement of Th17/IL-17 signaling pathway is also a possible mechanism of arthritis aggravation by *P. gingivalis*. These results also corroborate previous studies, including one from our group, using CIA model (de Aquino et al., 2014; Marchesan et al., 2013). A recent study demonstrated that the exacerbation of autoimmune arthritis in human leukocyte antigen-DR1 humanized C57Bl/6 mice under *P. gingivalis* infection was shown to be followed by a transitory increase of systemic Th17 population and in cervical lymph nodes. This same study also suggested a complementary mechanism because they detected the production of anti-citrullinated protein antibodies (ACPAs) – (Sandal et al., 2016); importantly, the generation of ACPAs was not detected in *P. gingivalis*- infected wild type C57Bl/6 mice. In this context of controversies, the present study showed that PD-induced arthritis aggravation in an AIA model is dependent on IL-17RA activation.

The AIA model is a relevant experimental model for studying arthritis due to the similar pathophysiology and immune events as those observed in human RA, in addition to its reproducibility (Brackertz, Mitchell, & Mackay, 1977; Pinto et al., 2010). Moreover, the contribution of antigen-specific CD4 + -derived mediators in triggering the inflammatory response in this model has been well described (Huber et al., 2006; Petrow, Thoss, Katenkamp, & Bräuer, 1996), and it is a

useful model for investigating the T cell-mediated molecular and cellular pathways involved in the PD and RA connection. Indeed, we have demonstrated the involvement of the Th17/IL-17 signalling pathway as a possible mechanism of AIA aggravation by *P. gingivalis* using this model. Importantly, the use of different experimental models to confirm a hypothesis is necessary, considering that each model has specific limitations and distinct targets of the innate and/or adaptive response, which can contribute to discrepancies in the final outcomes (Asquith et al., 2009).

Some animal studies have confirmed the opposite to that reported here, that periodontitis is exacerbated by experimentally-induced arthritis (Queiroz-Junior et al., 2012; Trombone et al., 2010). Studies have also shown that despite increased PD severity, the course of pristane-induced arthritis (Trombone et al., 2010) or AIA (Queiroz-Junior et al., 2012) were not affected by PD, which is different to our findings. A possible reason for this discrepancy could be the use of a different periodontal pathogen to induce PD in the study by Queiroz-Junior et al. (2012), who used *Agregatibacter Actinomycetemcomitans* instead of *P. gingivalis*. This is supported by a study that found that the course of experimental PD and the systemic immune response were altered by the presence of different strains of *P. gingivalis* (Marchesan et al., 2012). Recently, Corrêa et al. (2016) suggested that the induction of alveolar bone loss in mice subjected to AIA is only associated with changes in the oral microbiota (Corrêa et al., 2016). An additional explanation for these controversial findings is that the normal oral flora initially presented by the mice in these studies may have differed, which can influence the experimental PD outcome. It is also important to mention that other factors in the experimental design can vary between studies, including the immunisation protocol, challenge, dose of bacteria for oral inoculation and the bacterial strain, all of which can differ even if the same experimental model is used.

Importantly, in addition to the histopathological findings, we observed that oral *P. gingivalis* infection induced increased neutrophil infiltration into the articular space. As mentioned previously, the presence of neutrophils in the articular cavity is strongly associated with articular damage and hypernociception, and this has been shown to be dependent on Il-17 production in the inflamed joint (Lemos et al., 2009; Pinto et al., 2010). It is well established that IL-17 and TNF play a role in induction of the chemokine cascade that leads to neutrophil recruitment into the joint during arthritis (Lubberts et al., 2003). Consistent with this, we detected higher intra-articular levels of IL-17 and TNF in PD mice with AIA, in addition to increased neutrophil infiltration. Clinical data suggest that increased levels of TNF and IL-17 in RA patients are predictive of bone tissue destruction (Kirkham et al., 2006) as these cytokines play an important role in the neutrophil migration, induction of osteoclastogenesis and bone erosion characteristic of RA. We hypothesise that PD increases the levels of the proinflammatory TNF and IL-17 cytokines in the articular tissue at an earlier time point, due to the Th17 profile and aggravated RA observed in mice. Indeed, higher neutrophil infiltrate was detected in mice with AR and PD when compared to non-infected mice, a condition that was not present in IL-17RA-deficient mice. This suggests that aggravation of arthritis by *P. gingivalis* is dependent on functional IL-17/IL-17RA signalling.

We also demonstrated that *P. gingivalis*-induced periodontitis enhances mechanical hyperalgesia in experimentally-induced arthritis. Previous studies from our group have also demonstrated the involvement of TNF and IL-17 in the generation of articular hyperalgesia in an AIA model (Pinto et al., 2010). Therefore, we can infer that *P. gingivalis* increases mechanical hyperalgesia through local induction of the pronociceptive cytokines TNF and IL-17.

In addition to the local increase in IL-17 and neutrophil migration into joints, the frequency of Th17 cells was higher in the draining lymph nodes of ARPD mice, whereas Treg cell frequencies were not altered by *P. gingivalis* infection. Thus, we suggest that *P. gingivalis*-induced periodontitis can skew T helper cell differentiation toward the Th17 profile. This is in line with previous reports that have shown an increased Th17 population under *P. gingivalis* stimuli in both mice and humans (Cheng, Hughes, & Taams, 2014; Monteiro et al., 2009; Moutsopoulos et al., 2012). Indeed, clinical studies have shown the presence of Th17 cells in inflamed tissue, as well as increased local and serum levels of IL-17 during periodontitis (Adibrad et al., 2012; Awang et al., 2014; Cardoso et al., 2009). Moreover, local and systemic levels of IL-17 and the number of Th17 cells are reduced under periodontal treatment (Giannopoulou, Cappuyns, Cancela, Cionca, & Mombelli, 2012; Zhao et al., 2011). These data reinforce the influence of periodontitis on modulation of the Th17/IL-17 axis.

Maintenance of the chronic stimulus, as reported during chronic PD, may play a crucial role in the increased Th17 population and ultimately influence arthritis progression, as observed in our experimental model. In the current study, the effect of periodontal infection was probably not restricted to the oral cavity. It has been suggested that *P. gingivalis* and/or proinflammatory products from diseased sites may disseminate via blood or lymphatic circulation, leading to the priming or activation of distinct immune cells at distant sites (Hayashi, Gudino, Gibson, & Genco, 2010). We previously demonstrated that *P. gingivalis* activated bone marrow dendritic cells to produce T cells, affecting the secretion of proinflammatory cytokines in vitro (de Aquino et al., 2014). Therefore, it appears that the role of *P. gingivalis* in priming an innate immune response in dendritic cells (or other immune cells) may also contribute to T cell modulation and final aggravation of experimentally-induced arthritis. Moreover, previous reports have shown that modulation of Th17 by the gut microbiota can favour the development of arthritis in an experimental model (Abdollahi-Roodsaz et al., 2008; Wu et al., 2010). Interestingly, a recent study showed that oral *P. gingivalis* infection induces changes in the composition of the gut microbiota, an effect known to be associated with increased systemic inflammation (Arimatsu et al., 2014). Changes in the gut microbiota may represent an important mechanism by which *P. gingivalis* influences the host response at a distal site during systemic diseases. However, the effect of oral *P. gingivalis* infection (or other oral periodontal pathogens) on gut microbiota remains unclear, in addition to the other possible mechanisms by which *P. gingivalis* prime the host response at distal sites, affecting the arthritis outcome.

Additionally, further studies are necessary to elucidate whether *P. gingivalis* presents an arthritogenic antigen in addition to inducing a Th17 profile, and whether T cell cross-reactivity to *P. gingivalis*-derived epitopes and joint-derived antigens also participate in the aggravation of arthritis.

Taken together, our data demonstrate the pathogenic role of periodontal infection with *P. gingivalis* on the aggravation of arthritis mediated by increased Th17, articular production of IL-17 and activation of IL-17RA, which underlie the increase in neutrophil infiltration into the joints and ultimately affect arthritis progression.

These data support the importance of early periodontitis management in RA patients, as well as reinforcing the role of modulation of the Th17/IL17 axis in the AIA model as a mechanism for the influence of *P. gingivalis* on disease progression. This contributes to a better understanding of the etiological link between periodontitis and RA.

ACKNOWLEDGEMENTS

The authors would like to thank the excellent technical assistance of Ana Claudia G. C. Miranda, Tadeu Vieira and Giuliana Bertozi Francisco.

CONFLICTS OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

REFERENCES

Abdollahi-Roodsaz, S., Joosten, L. A., Koenders, M. I., Devesa, I., Roelofs, M. F., Radstake, T. R., … van den Berg, W. B. (2008). Stimulation of TLR2 and TLR4 differentially skews the balance of T cells in a mouse model of arthritis. *The Journal of Clinical Investigation*, *118*, 205–216. https://doi.org/10.1172/JCI32639

Adibrad, M., Deyhimi, P., Ganjalikhani Hakemi, M., Behfarnia, P., Shahabuei, M., & Rafiee, L. (2012). Signs of the presence of Th17 cells in chronic periodontal disease. *Journal of Periodontal Research*, *47*, 525–531. https://doi.org/10.1111/j.1600-0765.2011.01464.x

Al-Katma, M. K., Bissada, N. F., Bordeaux, J. M., Sue, J., & Askari, A. D. (2007). Control of periodontal infection reduces the severity of active rheumatoid arthritis. *Journal of Clinical Rheumatology : Practical Reports on Rheumatic & Musculoskeletal Diseases*, *13*, 134–137.

Al-Shammari, K. F., Al-Khabbaz, A. K., Al-Ansari, J. M., Neiva, R., & Wang, H. L. (2005). Risk indicators for tooth loss due to periodontal disease. *Journal of Periodontology*, *76*, 1910–1918.

de Aquino, S. G., Abdollahi-Roodsaz, S., Koenders, M. I., van de Loo, F. A., Pruijn, G. J., Marijnissen, R. J., … van den Berg, W. B. (2014). Periodontal pathogens directly promote autoimmune experimental arthritis by inducing a TLR2- and IL-1-driven Th17 response. *The Journal of Immunology*, *192*, 4103–4111. https://doi.org/10.4049/jimmunol.1301970

Arimatsu, K., Yamada, H., Miyazawa, H., Minagawa, T., Nakajima, M., Ryder, M. I., … Yamazaki, K. (2014). Oral pathobiont induces systemic inflammation and metabolic changes associated with alteration of gut microbiota. *Scientific Reports*, *4*, 4828. https://doi.org/10.1038/srep04828

Asquith, D. L., Miller, A. M., McInnes, I. B. & Liew, F. Y. (2009). Animal models of rheumatoid arthritis. *Eur J Immunol*, *39*, 2040–2044. doi:10.1002/eji.200939578.

Awang, R. A., Azman, R., Lappin, D. F., MacPherson, A., Riggio, M., Robertson, D., … Nile, C. (2014). Clinical associations between IL-17 family cytokines and periodontitis and potential differential roles for IL-17A and IL-17E in periodontal immunity. *Inflammation Research*, *63*, 1001–1012. https://doi.org/10.1007/s00011-014-0776-7

Bartold, P. M., Marino, V., Cantley, M., & Haynes, D. R. (2010). Effect of Porphyromonas gingivalis-induced inflammation on the development of rheumatoid arthritis. *Journal of Clinical Periodontology*, *37*, 405–411. https://doi.org/10.1111/j.1600-051X.2010.01552.x

Bıyıkoğlu, B., Buduneli, N., Aksu, K., Nalbantsoy, A., Lappin, D. F., Evrenosoğlu, E., & Kinane, D. F. (2013). Periodontal therapy in chronic periodontitis lowers gingival crevicular fluid interleukin-1beta and DAS28 in rheumatoid arthritis patients. *Rheumatology International*, *33*, 2607–2616. https://doi.org/10.1007/s00296-013-2781-5

Bozkurt, F. Y., Yetkin Ay, Z., Berker, E., Tepe, E., & Akkus, S. (2006). Anti-inflammatory cytokines in gingival crevicular fluid in patients with periodontitis and rheumatoid arthritis: A preliminary report. *Cytokine*, *35*, 180–185.

Brackertz, D., Mitchell, G. F., & Mackay, I. R. (1977). Antigen-induced arthritis in mice. I. Induction of arthritis in various strains of mice. *Arthritis and Rheumatism*, *20*, 841–850.

Brennan, F. M., & McInnes, I. B. (2008). Evidence that cytokines play a role in rheumatoid arthritis. *The Journal of Clinical Investigation*, *118*, 3537–3545. https://doi.org/10.1172/JCI36389

Cantley, M. D., Haynes, D. R., Marino, V., & Bartold, P. M. (2011). Pre-existing periodontitis exacerbates experimental arthritis in a mouse model. *Journal of Clinical Periodontology*, *38*, 532–541. https://doi.org/10.1111/j.1600-051X.2011.01714.x

Cardoso, C. R., Garlet, G. P., Crippa, G. E., Rosa, A. L., Júnior, W. M., Rossi, M. A., & Silva, J. S. (2009). Evidence of the presence of T helper type 17 cells in chronic lesions of human periodontal disease. *Oral Microbiology and Immunology*, *24*, 1–6. https://doi.org/10.1111/j.1399-302X.2008.00463.x

Cheng, W. C., Hughes, F. J., & Taams, L. S. (2014). The presence, function and regulation of IL-17 and Th17 cells in periodontitis. *Journal of Clinical Periodontology*, *41*, 541–549. https://doi.org/10.1111/jcpe.12238

Coat, J., Demoersman, J., Beuzit, S., Cornec, D., Devauchelle-Pensec, V., Saraux, A., & Pers, J. O. (2015). Anti-B lymphocyte immunotherapy is associated with improvement of periodontal status in subjects with rheumatoid arthritis. *Journal of Clinical Periodontology*, *42*, 817–823. https://doi.org/10.1111/jcpe.12433

Cope, A. P., Schulze-Koops, H., & Aringer, M. (2007). The central role of T cells in rheumatoid arthritis. *Clinical and Experimental Rheumatology*, *25*, S4–S11.

Corrêa, J. D., Saraiva, A. M., Queiroz-Junior, C. M., Madeira, M. F., Duarte, P. M., Teixeira, M. M., … da Silva, T. A. (2016). Arthritis-induced alveolar bone loss is associated with changes in the composition of oral microbiota. *Anaerobe*, *39*, 91–96. https://doi.org/10.1016/j.anaerobe.2016.03.006

Darveau, R. P., Pham, T. T., Lemley, K., Reife, R. A., Bainbridge, B. W., Coats, S. R., … Hajjar, A. M. (2004). Porphyromonas gingivalis lipopolysaccharide contains multiple lipid A species that functionally interact with both toll-like receptors 2 and 4. *Infection and Immunity*, *72*, 5041–5051.

Dudler, J., Renggli-Zulliger, N., Busso, N., Lotz, M., & So, A. (2000). Effect of interleukin 17 on proteoglycan degradation in murine knee joints. *Annals of the Rheumatic Diseases*, *59*, 529–532.

Farquharson, D., Butcher, J. P., & Culshaw, S. (2012). Periodontitis, Porphyromonas, and the pathogenesis of rheumatoid arthritis. *Mucosal Immunology*, *5*, 112–120. https://doi.org/10.1038/mi.2011.66

Giannopoulou, C., Cappuyns, I., Cancela, J., Cionca, N., & Mombelli, A. (2012). Effect of photodynamic therapy, diode laser, and deep scaling on cytokine and acute-phase protein levels in gingival crevicular fluid of residual periodontal pockets. *Journal of Periodontology*, *83*, 1018–1027. https://doi.org/10.1902/jop.2011.110281

Grespan, R., Fukada, S. Y., Lemos, H. P., Vieira, S. M., Napimoga, M. H., Teixeira, M. M., … Cunha, F. Q. (2008). CXCR2-specific chemokines

mediate leukotriene B4-dependent recruitment of neutrophils to inflamed joints in mice with antigen-induced arthritis. *Arthritis and Rheumatism*, *58*, 2030–2040.

van Hamburg, J. P., Asmawidjaja, P. S., Davelaar, N., Mus, A. M., Colin, E. M., Hazes, J. M., … Lubberts, E. (2011). Th17 cells, but not Th1 cells, from patients with early rheumatoid arthritis are potent inducers of matrix metalloproteinases and proinflammatory cytokines upon synovial fibroblast interaction, including autocrine interleukin-17A production. *Arthritis and Rheumatism*, *63*, 73–83. https://doi.org/10.1002/art.30093

Hayashi, C., Gudino, C. V., Gibson, F. C., & Genco, C. A. (2010). Review: Pathogen-induced inflammation at sites distant from oral infection: Bacterial persistence and induction of cell-specific innate immune inflammatory pathways. *Molecular Oral Microbiology*, *25*, 305–316. https://doi.org/10.1111/j.2041-1014.2010.00582.x

Hitchon, C. A., Chandad, F., Ferucci, E. D., Willemze, A., Ioan-Facsinay, A., van der Woude, D., … El-Gabalawy, H. S. (2010). Antibodies to porphyromonas gingivalis are associated with anticitrullinated protein antibodies in patients with rheumatoid arthritis and their relatives. *Journal of Rheumatology*, *37*, 1105–1112. https://doi.org/10.3899/jrheum.091323

Holt, S. C., Ebersole, J., Felton, J., Brunsvold, M., & Kornman, K. S. (1988). Implantation of Bacteroides gingivalis in nonhuman primates initiates progression of periodontitis. *Science*, *239*, 55–57.

van Holten, J., Reedquist, K., Sattonet-Roche, P., Smeets, T. J., Plater-Zyberk, C., Vervoordeldonk, M. J., & Tak, P. P. (2004). Treatment with recombinant interferon-beta reduces inflammation and slows cartilage destruction in the collagen-induced arthritis model of rheumatoid arthritis. *Arthritis Research & Therapy*, *6*, R239–R249.

Huber, L. C., Distler, O., Tarner, I., Gay, R. E., Gay, S., & Pap, T. (2006). Synovial fibroblasts: Key players in rheumatoid arthritis. *Rheumatology (Oxford)*, *45*, 669–675. https://doi.org/10.1093/rheumatology/kel065

Kirkham, B. W., Lassere, M. N., Edmonds, J. P., Juhasz, K. M., Bird, P. A., Lee, C. S., … Portek, I. J. (2006). Synovial membrane cytokine expression is predictive of joint damage progression in rheumatoid arthritis: A two-year prospective study (the DAMAGE study cohort). *Arthritis and Rheumatism*, *54*, 1122–1131.

Koenders, M. I., Marijnissen, R. J., Devesa, I., Lubberts, E., Joosten, L. A., Roth, J., … van den Berg, W. B. (2011). Tumor necrosis factor-interleukin-17 interplay induces S100A8, interleukin-1β, and matrix metalloproteinases, and drives irreversible cartilage destruction in murine arthritis: Rationale for combination treatment during arthritis. *Arthritis and Rheumatism*, *63*, 2329–2339. https://doi.org/10.1002/art.30418

Leech, M. T., & Bartold, P. M. (2015). The association between rheumatoid arthritis and periodontitis. *Best Practice & Research Clinical Rheumatology*, *29*, 189–201. https://doi.org/10.1016/j.berh.2015.03.001

Lemos, H. P., Grespan, R., Vieira, S. M., Cunha, T. M., Verri, W. A., Fernandes, K. S., … Cunha, F. Q. (2009). Prostaglandin mediates IL-23/IL-17-induced neutrophil migration in inflammation by inhibiting IL-12 and IFNgamma production. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 5954–5959. https://doi.org/10.1073/pnas.0812782106

Lubberts, E., van den Bersselaar, L., Oppers-Walgreen, B., Schwarzenberger, P., Coenen-de Roo, C. J., Kolls, J. K., … van den Berg, W. B. (2003). IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-kappa B ligand/osteoprotegerin balance. *The Journal of Immunology*, *170*, 2655–2662.

Marchesan, J. T., Gerow, E. A., Schaff, R., Taut, A. D., Shin, S. Y., Sugai, J., … Giannobile, W. V. (2013). Porphyromonas gingivalis oral infection exacerbates the development and severity of collagen-induced arthritis. *Arthritis Research & Therapy*, *15*, R186. https://doi.org/10.1186/ar4376

Marchesan, J. T., Morelli, T., Lundy, S. K., Jiao, Y., Lim, S., Inohara, N., … Giannobile, W. V. (2012). Divergence of the systemic immune response following oral infection with distinct strains of Porphyromonas gingivalis. *Molecular Oral Microbiology*, *27*, 483–495. https://doi.org/10.1111/omi.12001

Martinez-Martinez, R. E., Abud-Mendoza, C., Patino-Marin, N., Rizo-Rodriguez, J. C., Little, J. W., & Loyola-Rodriguez, J. P. (2009). Detection of periodontal bacterial DNA in serum and synovial fluid in refractory rheumatoid arthritis patients. *Journal of Clinical Periodontology*, *36*, 1004–1010.

Mikuls, T. R., Payne, J. B., Reinhardt, R. A., Thiele, G. M., Maziarz, E., Cannella, A. C., … O'Dell, J. R. (2009). Antibody responses to Porphyromonas gingivalis (P. gingivalis) in subjects with rheumatoid arthritis and periodontitis. *International Immunopharmacology*, *9*, 38–42.

Miossec, P., & Kolls, J. K. (2012). Targeting IL-17 and TH17 cells in chronic inflammation. *Nature Reviews. Drug discovery*, *11*, 763–776. https://doi.org/10.1038/nrd3794

Moen, K., Brun, J. G., Valen, M., Skartveit, L., Eribe, E. K., Olsen, I., & Jonsson, R. (2006). Synovial inflammation in active rheumatoid arthritis and psoriatic arthritis facilitates trapping of a variety of oral bacterial DNAs. *Clinical and Experimental Rheumatology*, *24*, 656–663.

Monteiro, A. C., Scovino, A., Raposo, S., Gaze, V. M., Cruz, C., Svensjo, E., … Scharfstein, J. (2009). Kinin danger signals proteolytically released by gingipain induce Fimbriae-specific IFN-gamma- and IL-17-producing T cells in mice infected intramucosally with Porphyromonas gingivalis. *The Journal of Immunology*, *183*, 3700–3711.

Moutsopoulos, N. M., Kling, H. M., Angelov, N., Jin, W., Palmer, R. J., Nares, S., … Wahl, S. M. (2012). Porphyromonas gingivalis promotes Th17 inducing pathways in chronic periodontitis. *Journal of Autoimmunity*, *39*, 294–303. https://doi.org/10.1016/j.jaut.2012.03.003

Ortiz, P., Bissada, N. F., Palomo, L., Han, Y. W., Al-Zahrani, M. S., Panneerselvam, A., & Askari, A. (2009). Periodontal therapy reduces the severity of active rheumatoid arthritis in patients treated with or without tumor necrosis factor inhibitors. *Journal of Periodontology*, *80*, 535–540.

de Pablo, P., Chapple, I. L., Buckley, C. D., & Dietrich, T. (2009). Periodontitis in systemic rheumatic diseases. *Nature Reviews Rheumatology*, *5*, 218–224.

Petrow, P. K., Thoss, K., Katenkamp, D., & Bräuer, R. (1996). Adoptive transfer of susceptibility to antigen-induced arthritis into severe combined immunodeficient (SCID) mice: Role of CD4 + and CD8 + T cells. *Immunological Investigations*, *25*, 341–353.

Pinto, L. G., Cunha, T. M., Vieira, S. M., Lemos, H. P., Verri, W. A. Jr, Cunha, F. Q., & Ferreira, S. H. (2010). IL-17 mediates articular hypernociception in antigen-induced arthritis in mice. *Pain*, *148*, 247–256. https://doi.org/10.1016/j.pain.2009.11.006

Queiroz-Junior, C. M., Madeira, M. F., Coelho, F. M., de Oliveira, C. R., Cândido, L. C., Garlet, G. P., … Silva, T. A. (2012). Experimental arthritis exacerbates Aggregatibacter actinomycetemcomitans-induced periodontitis in mice. *Journal of Clinical Periodontology*, *39*, 608–616. https://doi.org/10.1111/j.1600-051x.2012.01886.x

Reichert, S., Haffner, M., Keysser, G., Schafer, C., Stein, J. M., Schaller, H. G., … Schulz, S. (2013). Detection of oral bacterial DNA in synovial fluid. *Journal of Clinical Periodontology*, *40*, 591–598. https://doi.org/10.1111/jcpe.12102

Sandal, I., Karydis, A., Luo, J., Prislovsky, A., Whittington, K. B., Rosloniec, E. F., … Brand, D. D. (2016). Bone loss and aggravated autoimmune arthritis in HLA-DRβ1-bearing humanized mice following oral challenge with Porphyromonas gingivalis. *Arthritis Research & Therapy*, *18*, 249. https://doi.org/10.1186/s13075-016-1143-6

Trombone, A. P., Claudino, M., Colavite, P., de Assis, G. F., Avila-Campos, M. J., Silva, J. S., … Garlet, G. P. (2010). Periodontitis and arthritis interaction in mice involves a shared hyper-inflammatory genotype and functional immunological interferences. *Genes and Immunity*, *11*, 479–489. https://doi.org/10.1038/gene.2010.13

Wegner, N., Wait, R., Sroka, A., Eick, S., Nguyen, K. A., Lundberg, K., … Venables, P. J. (2010). Peptidylarginine deiminase from Porphyromonas gingivalis citrullinates human fibrinogen and α-enolase: Implications for autoimmunity in rheumatoid arthritis. *Arthritis and Rheumatism*, *62*, 2662–2672. https://doi.org/10.1002/art.27552

Wu, H. J., Ivanov, I. I., Darce, J., Hattori, K., Shima, T., Umesaki, Y., … Mathis, D. (2010). Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity*, *32*, 815–827. https://doi.org/10.1016/j.immuni.2010.06.001

Zhao, L., Zhou, Y., Xu, Y., Sun, Y., Li, L., & Chen, W. (2011). Effect of non-surgical periodontal therapy on the levels of Th17/Th1/Th2 cytokines and their transcription factors in Chinese chronic periodontitis patients. *Journal of Clinical Periodontology*, *38*, 509–516. https://doi.org/10.1111/j.1600-051X.2011.01712.x

How to cite this article: de Aquino SG, Talbot J, Sônego F, et al. The aggravation of arthritis by periodontitis is dependent of IL-17 receptor A activation. *J Clin Periodontol*. 2017;44:881–891. https://doi.org/10.1111/jcpe.12743