Commentary

Periodontitis and Rheumatoid Arthritis: What Do We Know?

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Background: Currently, in the field of rheumatology, there is much attention given towards the possible causality between periodontitis and rheumatoid arthritis (RA), specifically regarding the role of Porphyromonas gingivalis (Pg). This bacterium is unique, having a citrullinating enzyme. Antibodies against citrullinated proteins are rather specific for RA.

Methods: Because causality is ultimately tested in longitudinal cohort studies which currently do not exist for periodontitis and RA, this commentary applied Bradford Hill criteria on the existing literature to assess causality as the most likely interpretation of this association.

Conclusions: From an epidemiologic point of view, patients with RA have a higher incidence of periodontal disease than individuals without RA. In addition, there is a dose-response pattern in the association between the severity of periodontitis and RA disease activity. There are indications that periodontitis precedes RA, but there is no evidence yet available to show that Pg plays a direct role in this temporal relationship. The role of the unique characteristic of citrullination by Pg remains unexplained. However, in animal models, citrullination by Pg plays a distinct role in the development and aggravation of experimental arthritis. Although the role of Pg in RA remains speculative, a causative role for periodontitis as a chronic inflammatory disease caused by infectious agents in RA seems biologically plausible. J Periodontol 2015;86:1013-1019.

KEY WORDS
Arthritis, rheumatoid; causality; periodontitis; Porphyromonas gingivalis.

In the field of rheumatology, there is currently much attention given to the possible causality between periodontitis and rheumatoid arthritis (RA). Systemic inflammatory and infectious challenges have long been considered to be involved in triggering rheumatoid factor (RF), the first important biomarker for diagnosis and prediction of RA. Later, another autoantibody system, protein antibodies (ACPAs), was found to be more specific for RA. These antibodies can be present before the disease becomes symptomatic. Why ACPAs and RF are induced and their role in RA development are still unclear. The relationship between periodontitis and RA is not well understood. On one hand, systemic manifestation of increased “total inflammatory burden” through periodontitis has been documented. On the other hand, infection with Porphyromonas gingivalis (Pg) specifically has been suggested to play a role because of its unique capacity of protein citrullination.²³

Because causality is tested ultimately in longitudinal cohort studies that do not currently exist for periodontitis and RA, in this commentary, the Bradford Hill criteria are applied on existing literature to assess causality as the most likely interpretation of this association.

STRENGTH AND CONSISTENCY
Quantitative studies on the association between periodontitis and RA performed thus far are mostly case-control studies with a relatively small sample size. Nineteen of these studies, representing participants from different ethnic backgrounds, were included in a recent systematic review. It was concluded that patients with RA, compared with those without RA, have a significantly higher incidence of periodontitis and a higher number of missing teeth. A recent case-control study explored the degree to which the association is affected by shared genetic and/or environmental factors. After multivariable adjustments, including positivity for human leukocyte antigen-DRB1 shared epitope alleles, ever smoking, age, sex, race/ethnicity, body mass index, self-reported diabetes mellitus, marital status, presence of oral dryness, and education, the
incidence of periodontitis remained significantly higher in the ACPA seropositive patients with RA than in controls (odds ratio [OR] = 1.6). The association between periodontitis and RA is thus relatively consistent, but the strength of the association is uncertain.

**BIOLOGIC PLAUSIBILITY**

Certain genetic, hormonal, infectious, and environmental risk factors, such as smoking, can contribute to a susceptibility background against which RA can develop. In susceptible individuals, deregulation of the immune system occurs and can present itself with formation of autoantibodies, such as RF and ACPAs, that can be present before the disease becomes symptomatic. Whether and why progression to the symptomatic phase occurs is unknown, but theoretically a “second hit” could be necessary. In this “two-hit” model, the “first hit” is the induction of ACPA formation, and the second hit is the induction of arthritis and expression of citrullinated antigens in the inflamed joint.(Fig. 1). Inflammation may also be induced by infection with the periodontal pathogen _Pg_, which is unique in expressing a variant of the deiminating enzyme necessary for protein citrullination, peptidyl arginine deiminase (PAD). _Pg PAD_ (PPAD) is able to citrullinate endogenous and human proteins, thereby creating antigens that have been presumed to initiate the ACPA response in RA.

ACPAs in RA include antibodies against citrullinated histones. Histone citrullination is a common event during neutrophil activation and neutrophil death induced by different pathways, including apoptosis and neutrophil extracellular trap (NET) formation. NETs are increased in RA and are a source of citrullinated autoantigens. Neutrophils from patients with periodontitis have been shown to be hyper-reactive in terms of baseline, unstimulated generation and release of extracellular reactive oxygen. Because NET release is known to be dependent on production of extracellular reactive oxygen, periodontal disease may be associated with excessive production of NETs, i.e., in a process triggered initially by the response of neutrophils to plaque bacteria. High and concentrated levels of NET-associated molecules could lead to a localized chronic inflammatory response, potentially followed by an autoimmune response in genetically prone individuals.

Recently, Romero et al. identified a different citrullination pattern in PAD-expressing RA synovial fluid cells (neutrophils and monocytes), which they termed cellular hypercitrullination because of the broad spectrum of citrullination across the entire range of proteins. Cellular hypercitrullination is induced by two immune-mediated membranolytic pathways, mediated by perforin and the membrane attack complex, both leading to calcium influx. These data are supported by Neeli and Radic, who showed that, in human neutrophils, PAD4 induces histone citrullination in the presence of calcium ionophore. Up to now, it is unknown whether cellular hypercitrullination is present in inflamed periodontium.

**Mutual Exacerbation of Inflammatory Responses**

Another interpretation of the two-hit model is that of mutual exacerbation: periodontitis serves as the first hit, whereas an (unknown) arthritogenic hit induces RA (second hit) that leads, in susceptible individuals, to mutual exacerbation of the inflammatory responses mediating self-perpetuating tissue breakdown in both the joint and the periodontium (Fig. 1).

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**Figure 1.**

Hypothetical two-hit model proposed for the contribution of periodontitis to RA. Periodontitis and RA share certain genetic and environmental risk factors, including smoking and possibly infection with _Pg_. The named risk factors contribute to a susceptibility background against which periodontitis and/or RA can develop. In this two-hit model, the first hit is induction of ACPAs, possibly in the inflamed periodontium, and the second hit is induction of arthritis and expression of citrullinated antigens in the inflamed joint. Another possible sequence regarding the two-hit model is that of mutual exacerbation: periodontitis as a chronic inflammation, peptidyl arginine deiminase (PAD). _Pg PAD_ (PPAD) is able to citrullinate endogenous and human proteins, thereby creating antigens that have been presumed to initiate the ACPA response in RA.

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Neutrophils are the major cell type involved in periodontal inflammatory responses.\textsuperscript{19} Besides providing a source of citrullinated autoantigens, neutrophils can cause tissue breakdown through the release of degradative enzymes (e.g., matrix metalloproteinases) and cytotoxic substances, such as extracellular reactive oxygen. Neutrophils indirectly mediate destructive effects by chemotactic recruitment of T-helper 17 (Th17) cells. Th17 cells selectively produce the proinflammatory cytokine interleukin-17 (IL-17), which is crucial for host defense against extracellular pathogens.\textsuperscript{22} Uncontrolled Th17 activity has been implicated in joint inflammation and bone breakdown in RA, both at onset and in established disease.\textsuperscript{23,24} The presence of IL-17 and Th17 cells in human periodontitis may be associated with disease severity, possibly after activation of innate immune cells by \textit{Pg}.\textsuperscript{25} How this response regulates inflammation-mediated bone breakdown has not been fully elucidated (for illustration of possible mechanisms, see Hajishengallis\textsuperscript{26}).

In both periodontitis and RA, osteoclasts are activated predominantly by mechanisms dependent on upregulation of receptor activator of nuclear factor-\kappaB ligand (RANKL), a member of the tumor necrosis factor (TNF) cytokine family that is also implicated in RA.\textsuperscript{27} Mechanisms that underlie chronic joint inflammation in RA have not been clarified, although there is some evidence for both a T-cell dependent autoimmune mechanism and a more progressive fibroblast-mediated chronic inflammation.\textsuperscript{28} Th17 cells are recognized as effective B-cell helpers for antibody responses in inflammatory conditions.\textsuperscript{26} B-cells constitute, along with T-cells, a major source of membrane-bound and secreted RANKL in the periodontal lesions (for illustration, see Hajishengallis\textsuperscript{26}).

**TEMPORAL RELATIONSHIP**

In both interpretations of the two-hit model, periodontitis precedes RA. Commonly, more advanced forms of periodontitis are present at disease onset in patients with new-onset RA.\textsuperscript{29,30} A recent nationwide, population-based case-control study using longitudinal administrative data found an association between a history of periodontitis and newly diagnosed RA in Taiwan (OR = 1.2).\textsuperscript{31}

**SPECIFICITY REGARDING \textit{Pg}**

The \textit{PPAD} gene is highly conserved, ubiquitous in \textit{Pg}, and absent in \textit{Pg}-related species (unpublished observation; MJdS, JW, EB, AV, AJvW, G. Gabarrini [Center for Dentistry and Oral Hygiene, University of Groningen and University Medical Center Groningen, the Netherlands], K. Zhou [Department of Medical Microbiology, University of Groningen, University Medical Center Groningen], J.W.A Rossen [Department of Medical Microbiology, University of Groningen, University Medical Center Groningen], and J.M. van Dijl [Department of Medical Microbiology, University of Groningen, University Medical Center Groningen]). There were no indications that patients with RA carry a different \textit{PPAD} \textit{Pg} variant than patients without RA (unpublished observations; MJdS, JW, EB, AV, AJvW, G. Gabarrini [Center for Dentistry and Oral Hygiene, University of Groningen and University Medical Center Groningen, the Netherlands], K. Zhou [Department of Medical Microbiology, University of Groningen, University Medical Center Groningen], J.W.A Rossen [Department of Medical Microbiology, University of Groningen, University Medical Center Groningen], and J.M. van Dijl [Department of Medical Microbiology, University of Groningen, University Medical Center Groningen]). Studies on oral colonization by \textit{Pg} have shown no difference in subgingival \textit{Pg} distribution in patients with or without RA independent of periodontal status, detection techniques, and RA disease duration.\textsuperscript{6,29,30,32} However, DNA of periodontal pathogens has been detected in the synovial fluid of patients with RA.\textsuperscript{33} Of five different periodontal pathogens assessed, only DNA of \textit{Pg} was detected more frequently in the synovial fluid of patients with RA than in the synovial fluid of non-RA controls.\textsuperscript{34}

According to the hypothesis that \textit{Pg} contributes to ACPA production, the association of \textit{Pg} presence and serum ACPA levels has been assessed. In patients with new-onset RA, the subgingival presence of \textit{Pg} was not correlated with ACPA levels as determined using the diagnostic anti-cyclic citrullinated peptide 2 test.\textsuperscript{29} In patients with established RA, the presence of subgingival \textit{Pg} did not influence ACPA levels, but increased reactivity against several citrullinated peptides of fibrinogen, fillagrin, clusterin, histone 2B, and apolipoprotein was found when subgingival \textit{Pg} was present.\textsuperscript{6,32}

In patients with periodontitis but without RA, no differences in ACPA levels were found between \textit{Pg}-positive and \textit{Pg}-negative patients with periodontitis (unpublished observations MJdS, EB, AJvW, AV, JW, K.M.J. Janssen [Department of Oral and Maxillofacial Surgery, University of Groningen and University Medical Center Groningen, the Netherlands], F.A.C. de Kok [Department of Rheumatology and Clinical Immunology, University of Groningen and University Medical Center Groningen], J. Kraan [Department of Pulmonology, University of Groningen and University Medical Center Groningen], J. Altenburg [Department of Pulmonary Diseases, Medical Center Alkmaar, the Netherlands], M.K. Verheul [Department of Rheumatology, Leiden University Medical Center, the Netherlands], and L.A. Trouw [Department of Rheumatology, Leiden University Medical
Center), in contrast to Lappin et al., who found that serum ACPA levels in patients with periodontitis carrying subgingival \textit{Pg} were higher compared with patients with periodontitis without subgingival \textit{Pg}. The relatively low patient numbers in both studies and different detection techniques of \textit{Pg} and ACPA may account for this discrepancy.

Levels of serum anti-\textit{Pg} antibodies showed no differences in a large cohort of patients with RA and a cohort of osteoarthritis control patients. A study in Japanese patients with RA showed higher anti-\textit{Pg} antibody levels compared with age-, sex-, smoking status-, and periodontal condition-balanced healthy controls. Independent of \textit{Pg} distribution, a more robust antibody response was observed against \textit{Pg} in patients with established RA with severe periodontitis than in non-RA controls with the same periodontal status. The role of the antibody response in periodontitis is not fully understood, but it may be not protective. No differences in anti-\textit{Pg} antibody levels were found between ACPA and/or patients with RF seropositive arthralgia, who developed or did not develop RA within 2 years of follow-up.

In patients with established RA, a weak correlation between ACPA levels and anti-\textit{Pg} antibody levels has been found. Nevertheless, anti-\textit{Pg} antibody levels were found to be significantly higher in children with ACPA-positive juvenile idiopathic arthritis compared with children with ACPA-negative juvenile idiopathic arthritis, but no differences were noted for anti-\textit{Prevotella intermedia} and anti-\textit{Fusobacterium nucleatum} antibody levels.

**DOSE–RESPONSE RELATIONSHIP**

A dose–response pattern in the association between severity of periodontitis and RA development was found in a Taiwanese population based on longitudinal administrative data. Also, a large case-control study by Mikuls et al. revealed that the presence of periodontitis was associated with increased swollen joint counts and greater RA disease activity according to the 28-joint disease-activity score (DAS28). An association was shown between severity of periodontitis and severity of RA; patients with RA with severe periodontitis had significantly higher DAS28 scores than patients with RA with no or moderate periodontitis.

Experimental evidence in humans on the effect of periodontal treatment on RA disease activity is limited. Only four studies met the inclusion criteria according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines and were included in the recent meta-analysis by Kaur et al. on the effect of non-surgical periodontal therapy on RA disease activity measured by clinical (DAS28) and laboratory (erythrocyte sedimentation rate, TNFα, and C-reactive protein levels) parameters. These studies generally had a small sample size and a relatively short follow-up period (up to 6 months). Microbiology was not assessed, except for one study that measured serum anti-\textit{Pg} antibodies. Although these studies are considered preliminary and indicate the need for additional large-scale intervention studies, they reported a beneficial effect of periodontal therapy on laboratory RA parameters and clinical symptoms of RA.

**EXPERIMENTAL EVIDENCE**

The most widely studied model of RA is collagen-induced arthritis (CIA) in genetically susceptible mice. BALB/c mice with pre-existing periodontitis, induced by oral inoculations of \textit{Pg}, developed more severe CIA at a faster rate compared with CIA mice without periodontitis. Microcomputed tomography analysis of joint and periodontal bone loss provided evidence for a bidirectional relationship between periodontitis and arthritis; mice with CIA only showed alveolar bone loss, whereas mice with periodontitis only showed bone loss within radiocarpal joints. The severity of adjuvant arthritis in Dark Agouti rats was increased when there was a pre-existing extrasynovial chronic inflammatory lesion induced by subcutaneous sponges impregnated with heat-killed \textit{Pg}. No evidence of arthritis development occurred in Dark Agouti rats with a \textit{Pg} extrasynovial inflammatory lesion only; however, using computer-assisted morphometric analysis, periodontal bone loss after adjuvant arthritis induction was seen in Lewis rats as measured on defleshed jaws.

Experimental evidence for the involvement of periodontitis in the pathogenesis of RA via cellular immunity comes from a murine model of T-cell-dependent experimental arthritis. Periodontitis induced by oral inoculations with \textit{Pg} and \textit{Prevotella nigrescens} significantly aggravated the severity of CIA characterized by increased arthritic bone erosion in DBA/1 mice via induction of an antigen-specific Th17 response. A model in which experimental periodontitis and arthritis were co-induced in an inflammation-prone mouse strain using oral inoculations with \textit{Aggregatibacter actinomycetemcomitans} and \textit{Pg} in pristane-induced arthritis showed that co-induction in control mice did not alter the course of either periodontitis or arthritis. It was concluded that the interaction between periodontitis and arthritis in mice involves a shared hyperinflammatory genotype and functional interferences in innate and adaptive immune responses.

Experimental evidence for PPAD as a key player in the link between periodontitis and RA comes from aggravation of CIA in DBA/1 mice, which appeared...
dependent on the expression of PPAD. Increased serum ACPA levels were measured after infection with wild-type Pg W83 compared with mice infected with Pg W83 with PPAD deletion. Moreover, at the site of infection with the wild-type strain, higher levels of citrullinated proteins were found compared with the site of infection with the PPAD knock-out strain. These results were confirmed by Gully et al. using another murine model for experimental periodontitis and another Pg strain (W50); in BALB/c mice, the extent of CIA was significantly reduced in animals exposed to previous induction of periodontal disease through oral inoculation of a PPAD knock-out Pg W50 strain versus previous periodontal infection with the Pg W50 wild-type strain. Furthermore, serum ACPA tended to be lower in mice infected previously with PPAD-deficient Pg compared with ACPA levels in CIA mice with periodontitis induced by the wild-type strain.

**COHERENCE**

Animal models have shown that pre-existing periodontal infection (first hit) leads to exacerbation of arthritis after an arthritogenic hit (second hit). In the same animal models, a bidirectional relationship between experimental periodontitis and experimental arthritis existed, e.g., experimental arthritis leads to alveolar bone loss and experimental periodontitis leads to joint inflammation. This is in concordance with observations that newly diagnosed patients and patients with early RA have periodontitis more frequently and more periodontal attachment loss compared with controls. In human patients with periodontitis, joint inflammation has not been assessed systematically.

Mutual exacerbation of the inflammatory responses in animal experiments, in which both diseases coexisted, was shown to involve Th17-mediated immunity and to be dependent on a shared hyperinflammatory genotype. The correlation of RA disease activity with the severity of periodontitis in humans can probably be attributed to mutual exacerbation of inflammatory responses of both diseases. The importance of Th17-mediated immunity is acknowledged increasingly in human RA and periodontitis, whereas gene polymorphisms within the IL-1 gene cluster are associated with cytokine levels in patients with periodontitis and in patients with RA but not in healthy controls. The latter supports the hypothesis of a shared genetic background for cytokine profiles. IL-10 gene polymorphisms are also suggested to contribute to susceptibility for both RA and periodontitis. Three of several polymorphisms of IL-10 have been studied in some detail regarding RA susceptibility. Meta-analyses of 22 relevant studies on these IL-10 polymorphisms, all located in putative regulatory regions of the gene promoter, suggest that these IL-10 polymorphisms contribute to susceptibility of RA in European, Asian, and black populations. Recently, an IL-10 polymorphism, also located in the genetic region upstream of IL-10, was validated as a candidate gene in aggressive periodontitis in European patients.

Animal experiments have shown that there is an important role for PPAD in protein citrullination, ACPA formation, and development and aggravation of experimental arthritis. However, there are no differences in PPAD gene and endogenous citrullination patterns of Pg isolated from patients with or without RA (unpublished observations: MJdS, JW, EB, AV, AJvW, G. Gabarrini [Center for Dentistry and Oral Hygiene, University of Groningen and University Medical Center Groningen, the Netherlands], K. Zhou [Department of Medical Microbiology, University of Groningen, University Medical Center Groningen], J.W.A Rossen [Department of Medical Microbiology, University of Groningen, University Medical Center Groningen], and J.M. van Dijl [Department of Medical Microbiology, University of Groningen, University Medical Center Groningen]). Also, oral colonization by Pg is not different in patients with RA compared with patients without RA, independent of periodontal status, detection techniques, and RA disease duration. At this moment, the relevance of PPAD in priming autoimmunity and RA development in humans is not clear.

**ANALOGY**

Infections have been shown to be highly associated with the onset of systemic lupus erythematosus (SLE), a chronic destructive autoimmune disease characterized by immune deregulation and hyperproduction of different autoantibodies. Particularly, Epstein-Barr virus (EBV), parvovirus B19, retrovirus, and cytomegalovirus (CMV) infections might play a pathogenic role, but the etiopathogenesis of SLE is far from being completely elucidated. Viral (e.g., parvovirus B19, EBV, and CMV) and microbial (e.g., *Campylobacter*) infections play a role in acute arthritis, but the role of infection in the development of RA needs additional prospective controlled studies. Approximately 10% to 20% of patients with early RA have serologic evidence of recent infection, but no single infectious agent is predominant, which indicates that total infectious exposure can represent a risk factor that could trigger RA.

**CONCLUSIONS**

From an epidemiologic point of view, patients with RA have a higher incidence of periodontal disease than individuals without RA. In addition, there is a dose–response pattern in the association between the severity of periodontitis and RA disease activity.
There are indications that periodontitis precedes RA, but there is no evidence yet available to show that \( \text{Pg} \) plays a direct role in this temporal relationship. The role of the unique characteristic of citrullination by \( \text{Pg} \) remains unexplained. However, in animal models, periodontal pathogens and PPAD play a distinct role in the development and aggravation of experimental arthritis.

Although the role of periodontal pathogens in RA remains speculative, a causative role for periodontitis as a chronic inflammatory disease caused by infectious agents in RA seems biologically plausible. Considering the great variety in disease manifestation of both periodontitis and RA, a causal relationship, if one exists, may only be present between certain forms of periodontitis and RA.

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