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Pros and cons of causative association between periodontitis and rheumatoid arthritis

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1 | INTRODUCTION

In the context of a possible causative link between periodontal diseases and rheumatoid arthritis, it is worth noting that though periodontal diseases with associated dysbiotic microflora were prevalent in ancient populations as diverse as the Egyptians, prehistoric Scots, early pre-Columbian Americans, the Roman British, and medieval European populations,[1-6] bioarchaeological/paleontological studies have failed to reveal convincing evidence of rheumatoid arthritis. The first recognized description of rheumatoid arthritis dates to the beginning of the 19th century.[7] Owing to a lack of conclusive evidence, there are three hypotheses concerning the origin of rheumatoid arthritis: (a) it has an ancient origin; (b) it has a modern origin; and (c) it originated in the New World and was transmitted to the Old World after Columbus discovered the Americas. Therefore, if periodontal diseases (with pathogens dating back to the Neolithic period) underline development of rheumatoid arthritis, the first hypothesis would gain support in and lend credibility to an anecdotal report attributed to Hippocrates of the successful treatment of joint pain by extraction of bad teeth. Here, we present and critically discuss current research data, either supporting or negating the causative link between periodontal diseases and rheumatoid arthritis, in the context of the underlying inflammatory pathobiology of both diseases and possible involvement of periodontal pathogens.

2 | RHEUMATOID ARTHRITIS IN THE REFLECTION OF PERIODONTITIS?

Rheumatoid arthritis is a common, systemic autoimmune disease having a reported prevalence ranging from 0.5% to 1% in the adult population worldwide, with a female-to-male ratio of 3:1. This chronic disease is characterized by inflammation of the synovial lining of joints leading to the destruction of cartilage, erosion of bone, pain, and chronic disability. Rheumatoid arthritis usually affects joints in the hands, feet, and wrists, but progression of the disease may also affect the kidneys, skin, lungs, and liver. The disease is strongly associated with cardiovascular damage and other systemic complications, increasing mortality. The etiology of rheumatoid arthritis is complex, and its underlying pathogenic mechanisms are not fully elucidated. However, this disease shares striking similarities with periodontal diseases in its immunological, biological, and genetic background, suggesting a strong association between these two disorders.[8]

2.1 | Genetic and environmental risk factors

Numerous studies have identified genes implicated in the genetic predisposition to rheumatoid arthritis, the strongest of which is the shared epitope motif, a specific five amino acid sequence in the type II human leukocyte antigen (HLA) of the major histocompatibility complex class II protein. Several alleles are considered to contain the shared epitope motif, many of which are located in the *HLA-DRβ1* region. Expression of specific HLA-DRβ1 polymorphisms is associated with increased risk of developing rheumatoid arthritis, and individuals possessing one or more susceptibility alleles are associated with increased disease severity. By themselves, shared epitope motif alleles are thought to be the main genetic susceptibility factors.[9,10]

The possible role of *DRB1* alleles in alveolar bone resorption has recently been studied. Gehlot et al documented that transgenic mice carrying the human shared epitope motif–coding allele *DRB1*04:01*

have increased susceptibility to spontaneous periodontal inflammation and bone destruction.[11] Moreover, Sandal et al showed that exposure of the gingiva of HLA-DR1 humanized C57BL/6 mice to *Porphyromonas gingivalis* results in systemic inflammation with elevated level of cytokines, differentiation of T helper 17 cells (both in the peripheral blood and cervical lymph nodes), decrease of femoral bone density, and the generation of anti–citrullinated protein antibodies.[12]

Polymorphisms in other genes, including solute carrier family 22 member 3 (*SLC22A3*), runt-related transcription factor 2 (*RUNX2*), peptidylarginine deiminase *PADI4*, *TNFAIP3*, and *PTPN22*, may also confer susceptibility to rheumatoid arthritis. Interestingly, some of these may also contribute to the pathogenesis of periodontal diseases. For example, Schulz et al examined the impact of genetic variants in the *PTPN22* (rs2476601), *PADI4* (rs2240340), and *CTLA4* genes (rs3087243) on rheumatoid arthritis and periodontal diseases. They showed that the T allele of rs2476601 (*PTPN22*) is associated with a higher susceptibility to periodontal diseases within the rheumatoid arthritis group and is a significant biomarker of rheumatoid arthritis and periodontal diseases comorbidity.[13] Recently, a case-control study revealed that the *KCNQ1* rs2237892 allele is significantly associated with comorbidity of rheumatoid arthritis and periodontal diseases. The authors showed that patients with rheumatoid arthritis and chronic periodontitis carrying the T allele had increased disease severity and more inflammation than patients without caries did.[14]

Single-nucleotide polymorphisms of the human tumor necrosis factor-alpha gene *TNF-α*, such as rs1800629 (−308 promoter polymorphism; A/G), leads to increased expression of the inflammatory cytokine tumor necrosis factor-alpha, and it has also been connected to autoimmune diseases, including rheumatoid arthritis. Recently, the *TNF-α* rs1800629 polymorphism was also found to be significantly associated with periodontal diseases.[15]

Taken together, it seems that periodontal diseases and rheumatoid arthritis may share similar genetic risk factors. This complicates the search for a causal relationship, and it can be considered as an argument against a causative association between the diseases. Nevertheless, it needs to be considered that, though the shared epitope motif alleles *PTPN22* and, to a lesser degree, *PADI4* variants are strongly associated with rheumatoid arthritis, their impact on periodontal diseases is very weak.

The intriguing relationship between periodontal diseases and rheumatoid arthritis is further supported by related environmental risk factors. They can shape the immune response to bacterial infections or microbial dysbiosis in a manner that might break tolerance against host antigens. The process can occur on the mucosal surfaces—specifically the lungs, gut, and periodontium—confirming the observation that unhealthy diet, high alcohol consumption, poor oral hygiene, or cigarette smoking are risk factors of rheumatoid arthritis development.[16-19]

2.2 | Common pathophysiological mechanisms of inflammation

Although the etiologies of rheumatoid arthritis and periodontal diseases are different (one is autoimmune and the other is of an infectious nature), both diseases share several common features. Local chronic inflammation in the synovial compartment or gingival pockets is a typical clinical hallmark of both rheumatoid arthritis and periodontal diseases, respectively. In both cases, chronic inflammation is the result of inappropriate response of the innate and acquired immune systems at various stages of the disease. Both disorders are characterized by local accumulation of cytokines (eg, tumor necrosis factor-alpha, interleukin-1beta [IL-1β], interleukin-6 [IL-6], and monocyte chemoattractant protein-1), reactive oxygen species, nitric oxide, and lipid mediators, such as prostaglandin.[20] Uncontrolled inflammatory reactions result in increased infiltration of neutrophils, macrophages, and T and B-lymphocytes, which continuously migrate to affected sites in response to locally released chemokines. The persistence of activated phagocytes (macrophages, dendritic cells, and their secretion of inflammatory mediators, including chemokines) strongly promotes self-sustaining inflammation. Neutrophils play an important role in the pathogenesis of periodontal diseases and rheumatoid arthritis, constituting 80%-90% of the synovial fluid cells in rheumatoid arthritis patients and 80%-95% of the leukocytes present in the gingival crevicular fluid of periodontitis cases.[21] Their massive influx, combined with prolonged life span or impaired elimination, leads to their necrosis, degranulation, or formation of neutrophil extracellular traps. These neutrophil extracellular traps—which have been found in copious amounts in the gingival crevicular fluid, purulent crevicular exudates, and biopsies of the pocket epithelium from periodontitis patients[22]—lead to the increase and uncontrolled activity of enzymes (such as elastase, cathepsins, and proteinase 3) released from the secondary granules of neutrophils, along with excessive production of reactive oxygen species. Together, these enhance the destruction of the gingiva, periodontal ligament, and alveolar bone in patients with periodontal diseases, and of the subchondral bone and cartilage in patients with rheumatoid arthritis. The process is augmented by the activity of collagenolytic matrix metalloproteinases.[23] The upregulation of receptor activator of nuclear factor-κB ligand (RANKL) expression by fibroblasts and lymphocytes leads to osteoclast formation by macrophages in both the inflamed periodontium and joints.[24,25] T and B-cells have both been shown to be a major source of RANKL in the diseased synovium and periodontium.

Both rheumatoid arthritis and periodontal diseases are inflammatory and T helper cell–driven diseases. Recent evidence suggests that two types of T helper cells, T helper 1 and T helper 17, play a major role in rheumatoid arthritis and periodontal diseases pathogenesis.[26] The secretion of interleukin-17A (IL-17A) cytokines by T helper 17 cells activates a number of pathways, such as fibroblast-like synoviocytes, maturation and function of osteoclasts, and activation of neutrophils, macrophages, and B-cells.[27] Taken together, chronic inflammatory events and immunoregulatory imbalance eventually leads to the destruction of both the soft and hard tissues of the joint and alveolar bone in rheumatoid arthritis and periodontal diseases, respectively. For example, a local inflammatory reaction in the periodontal tissue or synovial microenvironment accompanies the systemic chronic inflammation. It has been reported

that individuals with periodontal diseases or rheumatoid arthritis suffer from underling systemic dysregulation of the inflammatory response, as manifested by elevated levels of tumor necrosis factor-alpha and IL-6 detected in the serum.[28]

Collectively, both diseases are characterized by a complex network of impaired interactions between components of the immune system, leading to a loss of homeostasis in the tissues and resulting in irreversible damage. The similarity of pathogenic mechanisms between periodontal diseases and rheumatoid arthritis is another complication when investigating the reciprocal comorbidity of these two diseases. Owing to the inflammatory nature of periodontal diseases and rheumatoid arthritis, each can exacerbate the other through the production of proinflammatory cytokines.

3 | ASSOCIATION BETWEEN PERIODONTITIS AND RHEUMATOID ARTHRITIS

Recent data from numerous clinical and epidemiological studies provide evidence for a bidirectional association between rheumatoid arthritis and periodontal diseases. Clinical trials show that patients with long-standing active rheumatoid arthritis have a substantially higher frequency of periodontal diseases than healthy subjects do.

On the other hand, some studies report that periodontitis is a risk factor for developing or even enhancing the severity of rheumatoid arthritis. In the following, we describe the most convincing reports from the clinical field, which are supported with data obtained using animal models.

3.1 | Clinical signs

Evaluation of periodontal status in rheumatoid arthritis patients revealed that periodontal diseases prevalence was at least twofold higher in rheumatoid arthritis patients, and that they had a worse periodontal condition than healthy controls.[29,30] The conclusion comes from the detailed dental examination assessed by probing pocket depth, clinical attachment level, and bleeding index determined at six sites per tooth, and the presence of supragingival plaque at four sites per tooth. The data presented by Eriksson et al revealed that the majority of rheumatoid arthritis patients tested (75%) had moderate or severe periodontitis, and the remainder had no or mild periodontitis,[30] strongly corroborating the results of earlier clinical studies.[31] These data were further supported by an observation of arthralgia in patients who had never been treated with antirheumatic drugs or glucocorticoids.[32] Moreover, extended analysis of systemic mediators of inflammation revealed increased levels of soluble CD30/tumor necrosis factor receptor superfamily member 8, interferon alpha-2, interleukin-19, interleukin-26, matrix metalloproteinase-1, glycoprotein 130/soluble IL-6 receptor beta, and soluble tumor necrosis factor receptor 1, in the serum or gingival crevicular fluid of rheumatoid arthritis patients.

Based on the hypothesis that periodontitis is a risk factor for the development of rheumatoid arthritis, it is important to examine the association between periodontitis and the risk of developing rheumatoid arthritis (pre–rheumatoid arthritis) or early signs of rheumatoid arthritis. A report by Bello-Gualtero et al studying 119 individuals with pre–rheumatoid arthritis showed significantly higher levels of plaque index, bleeding on probing, and severity of periodontal disease. In the study, periodontitis was associated with pre–rheumatoid arthritis but not with early signs of rheumatoid arthritis, and the appearance of anti–citrullinated protein antibodies preceded the onset of rheumatoid arthritis symptoms.[33] These findings were validated by Terao et al, who examined periodontal diseases status and the presence of anti–citrullinated protein antibodies and immunoglobulin M-rheumatoid factor in a cohort of 9554 adult healthy subjects.[34] These authors reported significant associations between periodontal diseases parameters, disease status, and level of anti–citrullinated protein antibodies, thereby supporting the involvement of anti–citrullinated protein antibodies production in periodontal diseases. Further, a study investigating the first-degree relatives of rheumatoid arthritis patients showed higher prevalence of periodontitis (79% versus 56%) in this group than in healthy subjects. Moreover, 15% of first-degree relatives had severe periodontitis.[35] This observation was confirmed by the increased severity of periodontitis in patients who were first-degree relatives, which was associated with seropositivity to anti–citrullinated protein antibodies. In individuals positive for anti–citrullinated protein antibodies, the mean plaque index, probing depth, bleeding on probing, clinical attachment level, and number of sites per person with probing depth >4 mm were significantly higher than in the group negative for anti–citrullinated protein antibodies. All subjects positive for anti–citrullinated protein antibodies had periodontitis, with 44.1% presenting with moderate periodontitis and 47.1% with severe periodontitis.[36]

The association of rheumatoid arthritis with periodontal diseases has also been examined in case-control studies enrolling patients with periodontal diseases. The data revealed that, compared with the general population, subjects with periodontal diseases are at an increased risk of developing rheumatoid arthritis. Moreover, the clinical course of periodontal diseases in rheumatoid arthritis patients is more severe and is independent of age, gender, ethnicity, or smoking history, when compared with non–rheumatoid arthritis individuals.[37,38] The association between periodontal diseases and rheumatoid arthritis is proposed to be based on the presence of anti–citrullinated protein antibodies and rheumatoid factor in the serum and gingiva of patients with periodontitis.[39] Using samples collected from 39 patients with periodontal diseases, Lappin et al showed that patients with untreated periodontitis had higher anti–citrullinated protein antibodies titers than healthy controls did.[40] Of note, the presence and titer of anti–citrullinated protein antibodies in serum is routinely measured using commercial kits utilizing immobilized cyclic citrullinated peptides as antigens. Owing to the limited evidence and inconsistent findings on whether periodontitis increases the risk for rheumatoid arthritis, Qiao et al performed a meta-analysis of 13 published studies including a total of 706 611 periodontitis patients

and 349 983 control subjects. The data from this meta-analysis indicated that patients with a periodontitis duration >5 years were more likely to develop rheumatoid arthritis and had a 69% greater risk for rheumatoid arthritis than people in the control group did.[41] This study showed that periodontitis represents a risk factor for rheumatoid arthritis.

3.2 | Microbiological data: periopathogens in rheumatoid arthritis patients

It is considered that the causal association between rheumatoid arthritis and periodontal diseases may be related, in great part, to periodontal pathogens. However, serological studies of antibodies specific for periopathogens yielded opposing observations when rheumatoid arthritis patients were compared with healthy controls. Some reports have shown that immunity to *P. gingivalis*, but not other periopathogens (*Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, and *Eikenella corrodens*), is significantly associated with the presence of rheumatoid arthritis–related autoantibodies in individuals at risk of rheumatoid arthritis, which suggests that infection with *P. gingivalis* may play a central role in the early loss of tolerance to self-antigens that occurs in the pathogenesis of rheumatoid arthritis.[42-44] Arvikar et al noted a similar observation in a subset of early signs of rheumatoid arthritis patients who had positive antibody responses to *P. gingivalis*. The responses correlated with anti–citrullinated protein antibodies reactivity. Compared with *P. gingivalis* antibody–negative patients, early signs of rheumatoid arthritis patients with positive *P. gingivalis* responses had more anti–cyclic citrullinated peptide (anti-CCP) antibody reactivity and significantly higher anti–cyclic citrullinated peptides levels, and the levels of anti-*P. gingivalis* antibodies correlated directly with anti–cyclic citrullinated peptides levels.[45] Conversely, another study found that anti-*P. gingivalis* antibody titers did not significantly differ between patients with rheumatoid arthritis and controls, nor did they significantly differ with anti–citrullinated protein antibodies, rheumatoid factor, or HLA shared epitope motif status.[46] In yet another study, immunoglobulin Gs (IgGs) specific for *P. intermedia*, *Prevotella melaninogenica*, and *Tannerella forsythia* were significantly higher in rheumatoid arthritis patients than in controls.[47] Moreover, a case-control study by Johansson and coworkers showed that anti-*P. gingivalis* antibody concentrations were significantly higher in rheumatoid arthritis patients than in controls, and were detectable years before onset of symptoms of rheumatoid arthritis.[48] A significant association was found between IgG against *P. gingivalis* and anti–citrullinated protein antibodies in individuals at risk for rheumatoid arthritis, and markers of rheumatoid arthritis activity in individuals with early signs of rheumatoid arthritis.[33] This observation was confirmed by Mankia and coworkers, who found an increased prevalence of periodontitis and *P. gingivalis* in anti–cyclic citrullinated protein antibody-positive at-risk individuals without arthritis.[49] Taken together, it seems that *P. gingivalis* positive-periodontitis is more likely to occur in individuals positive for anti–citrullinated protein antibodies without any arthritis, suggesting that periodontal diseases may precede rheumatoid arthritis. In a recent large population study in Korea, the authors

suggested that increasing periodontal indices could be used as a marker of rheumatoid arthritis development, and that anti-*P. gingivalis* antibody titer could inform about rheumatoid arthritis severity in patients suffering from periodontal diseases.[50] The discrepancy in these results, pertinent to the correlation between anti–*P. gingivalis* antibody levels and anti–citrullinated protein antibodies, prompted Bae and Lee to examine this issue using meta-analysis. The authors examined anti–*P. gingivalis* or anti–arginine-specific gingipain B antibody levels reported in 15 separate studies comprising a total of 3829 rheumatoid arthritis patients and 1239 controls. The meta-analysis demonstrated that the anti–*P. gingivalis* antibody is significantly higher in patients with rheumatoid arthritis, and that a positive relationship exists between anti–*P. gingivalis* antibody levels and anti–citrullinated protein antibodies.[51]

In addition to serological studies, several reports have described an association between the level of specific bacterial periopathogens in the subgingival biofilm and rheumatoid arthritis. For example, Schmickler et al reported higher *P. gingivalis* and *Fusobacterium nucleatum* levels in anti–cyclic citrullinated peptide–positive rheumatoid arthritis patients.[29] In a study conducted by Eriksson et al, a detailed analysis of plaque and saliva microbiota revealed an altered subgingival microbial profile between investigated groups of patients with periodontal diseases and rheumatoid arthritis. Rheumatoid arthritis patients with moderate or severe periodontitis had a significantly higher abundance of *Desulfobulbus* sp, *Prevotella* sp, *Bulleidia* sp, *Capnocytophaga* sp, *T. forsythia*, and a single nonidentified species in plaque than rheumatoid arthritis patients with no or mild periodontitis did. By contrast, *Prevotella oris* and *Porphyromonas* sp were more abundant in patients with no or mild periodontitis. The results showed that *P. gingivalis* was present more frequently (62%) in the moderate/severe periodontitis group than in the no/mild periodontitis group (50%). In saliva, there were no significant differences in the bacterial species detected in rheumatoid arthritis patients with or without periodontal diseases based on periodontal diagnosis.[30] By contrast, the number of *P. gingivalis* in tongue biofilm was significantly associated with the severity of rheumatoid arthritis disease expressed as Disease Activity Score-28. This observation suggests that the oral cavity microbiological status could play a role in the pathogenic mechanisms of rheumatoid arthritis–associated inflammation, leading to a more active disease state.[52]

4 | THERAPY

Because periodontal diseases and rheumatoid arthritis are associated, it is assumed that successful treatment of one disease should prevent or improve the outcomes of the other. We discuss the results of published studies of this in the following sections.

4.1 | Treatment of periodontal diseases and the influence on rheumatoid arthritis severity

The role of periodontal diseases in rheumatoid arthritis development is supported by evidence from studies that evaluated the

effects of periodontal treatment on rheumatoid arthritis severity in terms of the serum erythrocyte sedimentation rate, Disease Activity Score-28, C-reactive protein, and tumor necrosis factor-alpha levels.[37,53] Ribeiro et al revealed that erythrocyte sedimentation rate was reduced with no changes in the level of rheumatoid factor in rheumatoid arthritis patients who underwent full-mouth scaling and root planing.[54] By contrast, a recent report reported that scaling and root planing had no significant effects on erythrocyte sedimentation rate in patients with moderately active rheumatoid arthritis diagnosed with periodontal diseases.[55] Another study showed that scaling and root planing improved periodontal conditions in periodontal diseases in patients with and without rheumatoid arthritis. Moreover, the authors of this study suggested that, in patients with rheumatoid arthritis, eradication of *P. gingivalis* in conjunction with a high level oral hygiene can transiently decrease disease activity of rheumatoid arthritis.[56] Together, these data corroborate previous observations showing successful treatment of rheumatoid arthritis patients with antibiotics against bacterial anaerobic infections.[57]

4.2 | Treatment of rheumatoid arthritis and the influence on periodontal diseases severity

Among the clinical biomarkers currently used for the diagnosis of rheumatoid arthritis are rheumatoid factor, anti–perinuclear factor, anti–keratin antibodies, anti–filaggrin antibodies, and anti–cyclic citrullinated peptide antibodies, which are thought to be identical to anti–citrullinated protein antibodies. An investigation directed toward identifying new biologic markers revealed an association between increases in the levels of systemic proinflammatory mediators and rheumatoid arthritis, including IL-1, IL-6, interleukin-8, IL-17, interleukin-21, tumor necrosis factor-alpha, and granulocyte-macrophage colony-stimulating factor.[58] Therefore, patients suffering from rheumatoid arthritis are treated with anti-inflammatory drugs, which can also strongly influence their periodontal status. Among anti-inflammatory drugs routinely applied to treat rheumatoid arthritis are the inhibitors of tumor necrosis factor, such as infliximab and adalimumab (anti–tumor necrosis factor neutralization antibodies), and etanercept (an anti–tumor necrosis factor receptor antibody). Mayer et al evaluated the influence of infliximab on the periodontal health of patients with rheumatoid arthritis and the association between gingival crevicular fluid tumor necrosis factor-alpha levels and periodontal inflammatory parameters. They found that treatment of rheumatoid arthritis patients with infliximab significantly reduced the levels of tumor necrosis factor-alpha in gingival crevicular fluid and decreased periodontal inflammation.[59] Apparently, such treatment has no influence on the level of anti–*P. gingivalis* antibody, as shown by a recent study performed with 79 infliximab-treated rheumatoid arthritis patients.[60] Therefore, the effects of tumor necrosis factor inhibition on periodontal diseases parameters in rheumatoid arthritis patients remains an open question, but it is of interest given that tumor necrosis factor promotes osteoclastogenesis and inhibits osteoblastogenesis. The effect of IL-6 inhibition with rituximab or tocilizumab was also intensively studied.[61] Recent data demonstrated that treatment with tocilizumab (an anti–IL-6 receptor antibody) might not only improve clinical and biochemical rheumatoid arthritis–related parameters but also ameliorate periodontal diseases. The authors observed that tocilizumab therapy significantly improved the gingival index scores and decreased the number of sites with bleeding on probing after only 3 months, and the probing pocket depth decreased significantly after 6 months. The authors linked the positive clinical outcome to a decrease in the level of IL-6 in the periodontal microenvironment and reduced systemic inflammation.[62]

Among the drugs used to prevent rheumatoid arthritis development are conventional synthetic disease-modifying anti-rheumatic drugs. However, there are limited data on the influence of disease-modifying anti-rheumatic drugs on periodontal diseases. Some studies have reported no association between anti-rheumatic treatment and periodontal parameters,[63] whereas others have shown beneficial effects of disease-modifying anti-rheumatic drugs on periodontal clinical parameters following nonsurgical periodontal treatment.[64]

Collectively, the data from the majority of studies suggest that treatment of periodontitis and eradication of *P. gingivalis* could be a good approach to prevent rheumatoid arthritis. The same is apparent for treatment of rheumatoid arthritis, which has a beneficial effect on the clinical outcome of periodontal diseases. Together, these observations confirm the clinical association between periodontal diseases and rheumatoid arthritis, but they do not provide enough evidence for a causative relationship between these diseases. This is because both treatments target inflammation that is directly responsible for progression of both periodontal diseases and rheumatoid arthritis.

5 | MURINE MODELS

The association between periodontal diseases and rheumatoid arthritis revealed by clinical and epidemiological studies is strongly supported by animal studies. The involvement of periodontal pathogens in the development of rheumatoid arthritis is clearly documented by studies demonstrating that preexisting periodontitis exacerbates experimental arthritis in rat and mouse models.[65] Additionally, Bartold et al demonstrated that the presence of preexisting chronic inflammation exacerbated the development of adjuvant arthritis induced by injecting a mycobacterium cell wall in complete Freund's adjuvant into rats.[66] These data indicated that synovial inflammation, with pathobiology similar but not identical to periodontitis, promoted the development of experimental periodontal diseases. Further, several groups have confirmed the relationship between gingival inflammation and arthritis by infection with live *P. gingivalis* using the oral gavage model. In a collagen antibody–induced arthritis model, severe arthritis developed faster in infected mice than in controls.[65]

More recently, reports have been published implicating *P. gingivalis* as an etiologic factor contributing to disease severity in experimentally induced rheumatoid arthritis models.[12,67-69] A similar effect to infection with *P. gingivalis* was observed with other periodontal pathogens, including *Prevotella nigrescens*,[70] *F. nucleatum*, and *A. actinomycetemcomitans*.[71] Two studies have examined the effects of multispecies infection in an experimental model of rheumatoid arthritis, one including the use of *P. gingivalis*, *Treponema denticola*, and *T. forsythia*,[72] and the other using *P. gingivalis*, *F. nucleatum*, and *A. actinomycetemcomitans*.[71] In the latter study, mice inoculated with a mixture of all three pathobionts showed less alveolar bone loss than mice inoculated with *P. gingivalis* alone, suggesting that *F. nucleatum* and *A. actinomycetemcomitans* somehow attenuate *P. gingivalis*–induced alveolar bone loss. Their finding suggests that the interaction of bacteria-forming dysbiotic biofilm in periodontitis patients may have a strong influence on rheumatoid arthritis development and progression. Moreover, it was shown recently that therapeutic eradication of *P. gingivalis* with chlorhexidine and metronidazole reduced the incidence and alleviated the severity of collagen-induced arthritis comparable to methotrexate in a murine model of periodontitis.[73]

As rheumatoid arthritis is strongly associated with genetic risk factors, it is unsurprising that periopathogen infection has a significant impact on rheumatoid arthritis development in mice strains genetically susceptible for rheumatoid arthritis, such as SKG,[74] HLA-DR1 humanized C57BL/6 mice,[12] DBA/1JJmsSlc,[69] DBA/1J,[67] DBA/1 × 10.Q F1,[71] and B10.RIII.[72] Together, these results indicate that periodontitis may be the underlying condition facilitating rheumatoid arthritis development in genetically susceptible humans. This hypothesis is strongly supported by the work of Courbon et al, who demonstrated that rheumatoid arthritis is triggered by *P. gingivalis* oral infection alone without additional induction of experimental arthritis. The authors showed that *P. gingivalis* induced severe periodontal diseases and that this was accompanied by elevated levels of inflammatory mediators (IL-17 and CXC motif chemokine ligand 1) and antibodies against citrullinated proteins (anti–cyclic citrullinated peptide 2), with subsequent synovial inflammation and bone destruction. Eight months after oral *P. gingivalis* infection, the development of spontaneous arthritis in the rat ankle bone was slower than experimentally induced arthritis.[75] Despite differences in experimental design, these studies produced similar results and showed that mice with preexisting periodontitis developed more severe arthritis. Enhanced severity of arthritis was manifested by swelling and erythema in the fore and hind paws, a massive influx of leukocytes, elevated RANKL expression in the joints and periodontal tissues, the accumulation of osteoclasts, cartilage damage, and bone erosion, and increased serum C-reactive protein levels indicating systemic inflammation. Importantly, the aforementioned arthritic symptoms in animal models closely resemble the clinical signs of rheumatoid arthritis in humans.

By contrast, there are many reports showing that arthritis can influence the development of periodontitis, which confirms the hypothesis of a bidirectional relationship between these two diseases.

In one study, Ramamurthy et al demonstrated that induction of arthritis is associated with spontaneous loss of alveolar bone and increased levels of IL-1β, tumor necrosis factor-alpha, and metalloproteinases in the gingival tissues of an adjuvant arthritis animal model.[76] In another study, Trombone et al. documented that a hyperinflammatory genotype aggravates bacteria-induced periodontal diseases in a model of pristane-induced rheumatoid arthritis. The authors showed that pristane-induced rheumatoid arthritis resulted in alveolar bone loss, which was dependent on the presence of *A. actinomycetemcomitans* and *P. gingivalis*.[77] Further interesting data were obtained in a chronic antigen-induced arthritis model, whereby the authors observed the spontaneous development of periodontal diseases. They found that antigen-induced arthritis resulted in severe alveolar bone loss, migration of osteoclasts, and release of proinflammatory cytokines in the maxillae.[78]

Taken together, animal models of periodontal diseases and rheumatoid arthritis provide the most conclusive evidence that not only can periodontitis induce rheumatoid arthritis but that the reverse is also true. This supports the hypothesis that inflammation is the most important causative factor for both rheumatoid arthritis and periodontal diseases (Table 1).

6 | MOLECULAR MECHANISM OF PERIODONTAL DISEASES-INDUCED RHEUMATOID ARTHRITIS

As most of the research in the field has focused on the infectious etiology of rheumatoid arthritis, including periodontitis, scientists are actively searching for the causative role of bacteria in the initiation and/or progression of rheumatoid arthritis. Autoantibodies are drivers of damaging immune responses and are strongly and specifically associated with autoimmune diseases. In the case of rheumatoid arthritis, the most important autoantibodies are directed against posttranslationally modified proteins, which are often produced by the inflamed tissues. The most common modifications include citrullination, carbamylation, or proteolytic fragmentation of proteins. In genetically susceptible individuals, these can be recognized as epitopes by the aberrant humoral immune response. Therefore, elucidation of the immunotolerance breakdown mechanism is crucial in understanding the pathology of autoimmune diseases, including rheumatoid arthritis. In the case of rheumatoid arthritis, despite very extensive research, the pathological conditions and anatomical locations where immunotolerance breakdown against citrullinated and carbamylated epitopes occurs are still disputable.

6.1 | Posttranslational modified proteins

An autoimmune reaction in rheumatoid arthritis patients is characterized by generation of anti–citrullinated protein antibodies, which is detected long before the clinical onset of the disease.[79] This antibody is both highly specific and sensitive for the diagnosis of

TABLE 1 A compilation of data showing the pros and cons of causative relationship between periodontitis and rheumatoid arthritis

Pros	Cons
• Periodontal diseases patients are at greater risk for rheumatoid arthritis than healthy individuals[41]	• Rheumatoid arthritis risk alleles within the human leukocyte antigen (particularly HLA-DRB1) predispose to periodontal diseases[12]
• Inflammatory nature of both diseases; bidirectional exacerbation through inflammatory mediators without causative relationship[20,23-25]	• Polymorphism of *PTPN22*, *KCNQ1*, and *TNF-α* is proposed as a marker for rheumatoid arthritis and periodontal diseases comorbidity[13-15]
• Periodontal diseases prevalence and severity is higher in individuals with pre–rheumatoid arthritis, first-degree relatives, and rheumatoid arthritis patients[29,30,33-35]	• Anti–*P. gingivalis* antibody titer did not differ between rheumatoid arthritis and healthy control[46]
• Significantly higher level of antibodies specific for periodontal pathogens in rheumatoid arthritis patients, including individuals at early stages of rheumatoid arthritis[33,42-45,47,48]	• The rheumatoid arthritis treatment has no influence on anti–*P. gingivalis* antibodies level[60]
• Association of periodontal diseases clinical parameters and anti*Porphyromonas gingivalis* antibodies with anti–citrullinated protein antibodies in rheumatoid arthritis and individuals at high risk of rheumatoid arthritis development[49,50]	• *A. actinomycetemcomitans* is considered a pathogen of rare aggressive forms of periodontal diseases
• Increased prevalence of periodontal diseases and *P. gingivalis* in anti–citrullinated protein antibodies–positive rheumatoid arthritis patients and individuals at risk of rheumatoid arthritis development[33,49]	• *P. gingivalis*–derived citrullinated peptides are not recognized by anti–citrullinated protein antibodies in early rheumatoid arthritis[138]
• Periodontal diseases treatment and eradication of *P. gingivalis* improved rheumatoid arthritis status,[54,56] whereas rheumatoid arthritis treatment ameliorated severity of chronic periodontal diseases[59,62]	• Preexisting chronic inflammation induced by other than periopathogens factors exacerbates the development of adjuvant arthritis[66]
• Preexisting periodontitis exacerbates experimental arthritis[65,71,72]	• Spontaneous alveolar bone loss in the adjuvant arthritis model[76]
• The inflamed periodontium is a source of posttranslational modifications proteins; *Aggregatibacter actinomycetemcomitans* is a potent stimulator of peptidylarginine deiminases; *P. gingivalis*–derived peptidylarginine deiminase citrullinates bacterial and host proteins[118,130,135]	
• Citrullinated *P. gingivalis* proteins and peptides react with anti–citrullinated protein antibodies[135,140,141]	
• *P. gingivalis* and *Prevotella nigrescens* promote arthritis progression via interleukin-17 signaling[67,70]	
• Molecular mimicry of bacterial enolase, heat shock protein 60 and arginine-specific gingipain A; bacterial proteins are recognized by antibodies from rheumatoid arthritis patients[145,148,150]	

Note: A a, Aggregatibacter actinomycetemcomitans; PADs, peptidylarginine deiminases; PD, periodontal disease; PPAD, *P gingivalis* PAD; PTMs, posttranslational modifications; RA, rheumatoid arthritis.

rheumatoid arthritis.[80] It is produced in response to the increased level of citrullinated proteins, which are considered to be the main factors causing the breakdown of tolerance observed during rheumatoid arthritis development. Of note, an inflamed periodontium is reported to be a rich source of citrullinated proteins.[81,82] These proteins have been found in the gingiva, gingival crevicular fluid, and saliva of periodontal diseases patients and include citrullinated histones, which are targets of autoantibodies in rheumatoid arthritis patients.[83-85]

There are several reports indicating that anti–carbamylated protein antibodies are also present in the sera of patients with rheumatoid arthritis.[86,87] Because the level of this autoantibody is predictive of joint damage, it has also been proposed as a diagnostic marker for rheumatoid arthritis.[87,88] Studies examining experimental arthritis have also shown that the appearance of anti–carbamylated protein antibodies in the sera precedes the onset of rheumatoid arthritis.[89,90] Of note is that inflamed human periodontal tissue constitutes a significant source of carbamylated proteins.[82] It is tempting to propose that carbamylation in the inflamed periodontium constitutes a plausible mechanism for the generation of antigens involved in the development and progression of rheumatoid arthritis.

Apart from carbamylation and citrullination, malondialdehyde-acetaldehyde adduct formation is significantly increased in rheumatoid arthritis patients.[91] IgG and immunoglobulin A anti–malondialdehyde-acetaldehyde autoantibodies were detected in rheumatoid arthritis patients prior to clinical diagnosis. As they appear later in the preclinical course than anti–citrullinated protein antibodies or rheumatoid factor do, the authors proposed that malondialdehyde-acetaldehyde adduct formation and anti–malondialdehyde-acetaldehyde immune responses could play a role in the transition from subclinical autoimmunity to clinically apparent arthritis.[92] Notably, malondialdehyde-acetaldehyde–modified proteins have been found in inflamed periodontal tissue as they are generated as a result of inflammation-associated oxidative stress. However, because current data suggest that anti–malondialdehyde-acetaldehyde autoantibodies may play a role in the development of rheumatoid arthritis, further investigations are needed.[93]

All posttranslational modifications described herein can initiate the generation of autoantibodies, thus promoting the breakdown of immune tolerance, which apparently occurs outside of the synovium on mucosal surfaces. Therefore, in the following we focus on the inflammatory reaction in the periodontium with emphasis on oral

pathogens, which may trigger the production of disease-specific autoantibodies and arthritis development in susceptible individuals.

6.2 | Inflammation in the periodontium as a source of autoantigens

The chronic inflammation in periodontal diseases constitutes a perfect niche for the generation of neo-epitopes due to the local release and/or accumulation of protein substrates and enzymes that catalyze posttranslational modifications.

6.2.1 | Soluble mediators of inflammation and enzymes

A complex network of inflammatory mediators, comprising cytokines, complement molecules, enzymes (proteases and peptidylarginine deiminases) and alarmins, is involved in the immunological processes that promote autoimmunity and ultimately tissue destruction in rheumatoid arthritis. Some of these play roles in the recruitment of leukocytes or the release of enzymes, whereas some directly modify the antigens released from dying cells. Those factors coordinate the extracellular conversion of proteins into immunodominant epitopes, known as remnant epitopes. In the following, we describe the most convincing data from this field, linking the etiology of rheumatoid arthritis with periodontal diseases.

Anaphylatoxin C5a is the most important factor in the complement system and plays a crucial role in the inflammation underlying the pathogenesis of rheumatoid arthritis and periodontal diseases.[94] The C5a molecule was identified in alveolar and synovial tissue.[95] Inhibition of the C5a-C5aR axis limits the inflammation and bone destruction observed in murine models of rheumatoid arthritis and periodontal diseases, and C5aR-deficient SKG mice with reduced arthritis.[96-98] Furthermore, arginine-specific gingipains very efficiently release the active C5a molecule directly from C5.[99] The involvement of C5a in the progression of rheumatoid arthritis infection was investigated by Munenaga et al,[100] which revealed that neutralization antibody against C5a suppresses osteoclast differentiation when mice with experimental arthritis are orally infected with *P. gingivalis*. A potential role for *P. gingivalis* in the activation of the C5a signaling pathway and rheumatoid arthritis pathogenicity is additionally supported by the fact that C5a promotes the development of T helper 17 cells. Homeostasis in the periodontium is considered to be dependent on the T-cell population, as a proper balance of T helper 1, T helper 2, and T helper 17 regulates the immune events that prevent bone destruction. Among T-cells, T helper 17 seems to play a unique role in cytokine secretion, including IL-17, which promotes osteoclast differentiation and the development of bone erosions. Using a murine model, *P. gingivalis* and *P. nigrescens* oral infection was shown to shift the balance of T-cells into a T helper 17 population. The T helper 17–driven response was manifested by elevated serum levels of IL-17 and interferon-gamma, increased osteoclast numbers in

joints, and enhanced arthritis progression and development.[67,70] As the levels of IL-17 induced by periodontal pathogens directly correlated with the intensity of arthritic bone erosion, the authors suggested that interleukin-7 has a potential role in rheumatoid arthritis pathology.[70] The role of IL-17 signaling in arthritis progression in the presence of *P. gingivalis* was confirmed using IL-17 receptor A–knockout mice.[101]

The expression of matrix metalloproteinases is highly upregulated in rheumatoid arthritis and periodontal diseases. Matrix metalloproteinases are essential for connective tissue homeostatic turnover, but they are also responsible for tissue damage if not properly regulated. This happens at chronic inflammatory sites where the unrestrained activity of matrix metalloproteinases leads to the accumulation of a range of protein-degradation products, derived from collagen types I, II, and III, vimentin, and C-reactive protein.[102] In the case of gingival crevicular fluid and synovial fluid, it was shown that matrix metalloproteinase-8 and matrix metalloproteinase-9 released from accumulating neutrophils degrade the collagen fibers that generate neo-epitopes.[103] Moreover, in the gingival crevicular fluid of periodontal sites infected with *P. gingivalis*, lysine-specific gingipain cleaves human IgG in vivo, releasing antigen-binding fragments that form remnant epitopes.[104]

Impaired elimination of leukocytes from the inflammatory milieu leads to their necrosis, followed by the release of cellular contents. Among the factors released are a group of molecules constituting endogenous damage–associated molecular patterns, including alarmins. Besides being spontaneously released, alarmins can be actively secreted by leukocytes, and oral and salivary tissue. To this end, it was shown that *P. gingivalis*, *T. forsythia*, *T. denticola*, and *F. nucleatum* induce the release of alarmins, including IL-1, adenosine triphosphate, heat shock protein 60, fibronectin, and high mobility group box protein 1 (HMGB1), from human cells.[105-107]

The role of high mobility group box protein 1 has been further studied. The concentration of this alarmin is elevated in gingival crevicular fluid, and large numbers of cells in the gingiva of periodontal diseases patients are high mobility group box protein 1 positive. High mobility group box protein 1 promotes the differentiation of osteoclasts as well as the activation of dendritic cells, T-cells, and endothelial cells. Systemic administration of anti–high mobility group box protein 1 inhibited periodontal inflammation and alveolar bone loss in an oral gavage model of periodontitis,[108] highlighting a potential role for this alarmin in the pathology of periodontal diseases and rheumatoid arthritis.

6.2.2 | Neutrophils and peptidylarginine deiminases

The conversion of positively charged arginine to the neutral citrulline residue is catalyzed by peptidylarginine deiminases. In humans, there are five peptidylarginine deiminases, each of which is distributed in different tissues. They play important physiological roles, including control of the immune system, skin keratinization, and the regulation of global gene expression.[109] Under inflammatory

conditions, peptidylarginine deiminase activity is considerably increased with pathological effects. It was shown that accelerated necrosis, or formation of neutrophil extracellular traps, constitute the main source of active peptidylarginine deiminase 4 enzyme.[110,111] Therefore, neutrophils are suggested to play an essential role in immune tolerance breakdown and anti–citrullinated protein antibodies generation, which eventually triggers the development of clinical rheumatoid arthritis.[112,113]

Periodontitis is characterized by the accumulation of high numbers of neutrophils, as well as their overactivity and incomplete removal by efferocytosis from periodontal tissues. The uncontrolled activity of neutrophil proteases and excessive release of reactive oxygen species contribute to the destruction of periodontal soft tissue and damage of organic components of the alveolar bone.[114] Neutrophil extracellular traps have been found in gingival crevicular fluid, purulent crevicular exudates, and biopsies of the pocket epithelium of periodontitis patients.[22,115] The abundant neutrophil extracellular nets formation is likely due to neutrophils interacting with the bacteria in periodontal pockets.[116] Recently, it was shown that *P. gingivalis* plays a crucial role in neutrophil extracellular trap formation as gingipains can directly induce neutrophil extracellular trap generation in vitro by hijacking the protease-activated receptor-2 signaling pathway.[117] On the other hand, *A. actinomycetemcomitans* was identified as the potent stimulator of peptidylarginine deiminase activity in neutrophils.[118] Among different periodontal pathogens and oral commensals, only *A. actinomycetemcomitans* has been reported to hijack activity of host peptidylarginine deiminases and induce hypercitrullination of proteins in the inflamed periodontium and rheumatoid joint. The process depends on secreted leukotoxin A, a pore-forming toxin that induces calcium influx, and subsequent hyperactivation of peptidylarginine deiminase enzymes in the neutrophil. Exposure to leukotoxic *A. actinomycetemcomitans* strains is more prevalent both in periodontitis and in rheumatoid arthritis patients than in healthy individuals and when associated with anti–citrullinated protein antibodies.[118] Contradictory results were published by Engström et al, who found no correlation between the increased level of citrullinated proteins and peptidylarginine deiminase 2 or peptidylarginine deiminase 4 enzymes in the gingival tissue of periodontal diseases patients, or the presence of periodontal pathogens, including *P. gingivalis* and *A. actinomycetemcomitans*.[119] These results argue against a role for neutrophils, as well as dysbiotic bacteria, in the generation of citrullinated proteins. Another mechanism deserving attention is protein carbamylation facilitated by myeloperoxidase of neutrophils infiltrating in large numbers into the inflamed periodontium.[86] Myeloperoxidase catalyzes the oxidation of thiocyanate in the presence of hydrogen peroxide to cyanate, which spontaneously reacts with N-terminal α-amino groups and ε-amino of lysine to generate homocitruline (ε-carbamyl-lysine) in proteins and peptides. Thus, the breakdown of immunotolerance in rheumatoid arthritis patients is still ill-defined and requires more investigation.

Altogether, the uncontrolled inflammatory reaction, combined with a high degree of destruction observed in periodontal tissues, makes the mechanism of the bystander activation in periodontal diseases–induced rheumatoid arthritis development quite plausible.

# 7 | ORAL-GUT MICROBIOME AXIS AND RHEUMATOID ARTHRITIS PATHOGENESIS

The gut microbiome has been intensively studied because, among many other functions, it contributes to the development and maintenance of the immune system. Therefore, there is no surprise that dysbiosis in this microbial community is linked to autoimmune diseases, including rheumatoid arthritis.[17] In the case of rheumatoid arthritis, the focus of investigation is on the *Prevotella* species, especially *Prevotella copri*. In a study published in 2013, *P. copri* was abundantly detected in fecal samples of new-onset untreated rheumatoid arthritis patients. The abundance of *P. copri* strongly correlated with disease severity, and was associated with a decrease in Bacteroides and a deficit of allegedly beneficial microbes in new-onset untreated rheumatoid arthritis subjects.[120] Until now, several reports have confirmed this ground-breaking finding strongly suggesting *P. copri* as a causative pathobiont in rheumatoid arthritis. Colonization of germ-free mice with *Prevotella copri* alone induced arthritis in a T helper 17 cell–dependent manner that closely resembled clinical rheumatoid arthritis in humans.[121] The derived peptides form a 27 kDa protein of *P. copri*, which was identified in complex with HLA-DR in the synovial environment and stimulated T helper 1 responses in the new-onset untreated rheumatoid arthritis patients.[122,123] Finally, it was shown that *P. copri is* enriched in the gut microbiota before the onset of the clinical rheumatoid arthritis in the new-onset untreated rheumatoid arthritis cohort.[124] Recently, a metagenome-wide association study revealed a high abundance of other species belonging to the *Prevotella* genus, including *Prevotella denticola*, *Prevotella marshii*, *Prevotella disiens*, *Prevotella corporis*, and *Prevotella amnii*, in rheumatoid arthritis patients but not in healthy controls.[125] Interestingly, *P. gingivalis* was also found in the feces of rheumatoid arthritis patients, but at relatively low abundance. Nevertheless, *P. gingivalis* abundance was positively correlated with *Prevotella* spp,[126] suggesting cooperation between *P. gingivalis* and *Prevotella* spp in the gut microbiome of rheumatoid arthritis patients. This finding adds an interesting twist to the interpretation of the mechanism of *P. gingivalis*–induced rheumatoid arthritis in mice models. Instead of exerting a direct effect on immunotolerance breakdown, *P. gingivalis* may initiate a chain of events that lead to the generation of anti–citrullinated protein antibodies and finally clinical rheumatoid arthritis, via changes in gut microflora that alter the gut immune system. Oral gavage of *P. gingivalis* impairs the function of the gut barrier and provokes dysbiosis in the gut microbiome through induced metabolic changes and endotoxemia.[127,128] Immunologically, this is manifested by increased IL-17 levels in the sera and conditioned media of cultured lymphocyte fractions obtained from the spleen, Peyer's patches, mesenteric lymph

nodes, and inguinal lymph nodes of mice orally administered with *P. gingivalis*. Furthermore, *P. gingivalis* gavage caused a significant increase in the proportion of T helper 17 cells among mesenteric lymphocytes and impacted the composition of the gut microbiome. A similar response might happen in humans if *P. gingivalis* is swallowed and colonizes the gut. Acting as the keystone pathobiont, it may affect the gut microbiota composition and immune system despite its very low relative abundance, thus indirectly contributing to development of rheumatoid arthritis.

7.1 | The direct effect of periodontal pathogens on immunotolerance breakdown

7.1.1 | Enzymatic mimicry: *P. gingivalis*–derived peptidylarginine deiminase

Citrullination of key rheumatoid arthritis human antigens is catalyzed by a unique peptidylarginine deiminase enzyme: *P. gingivalis*–derived peptidylarginine deiminase. Since the first report describing
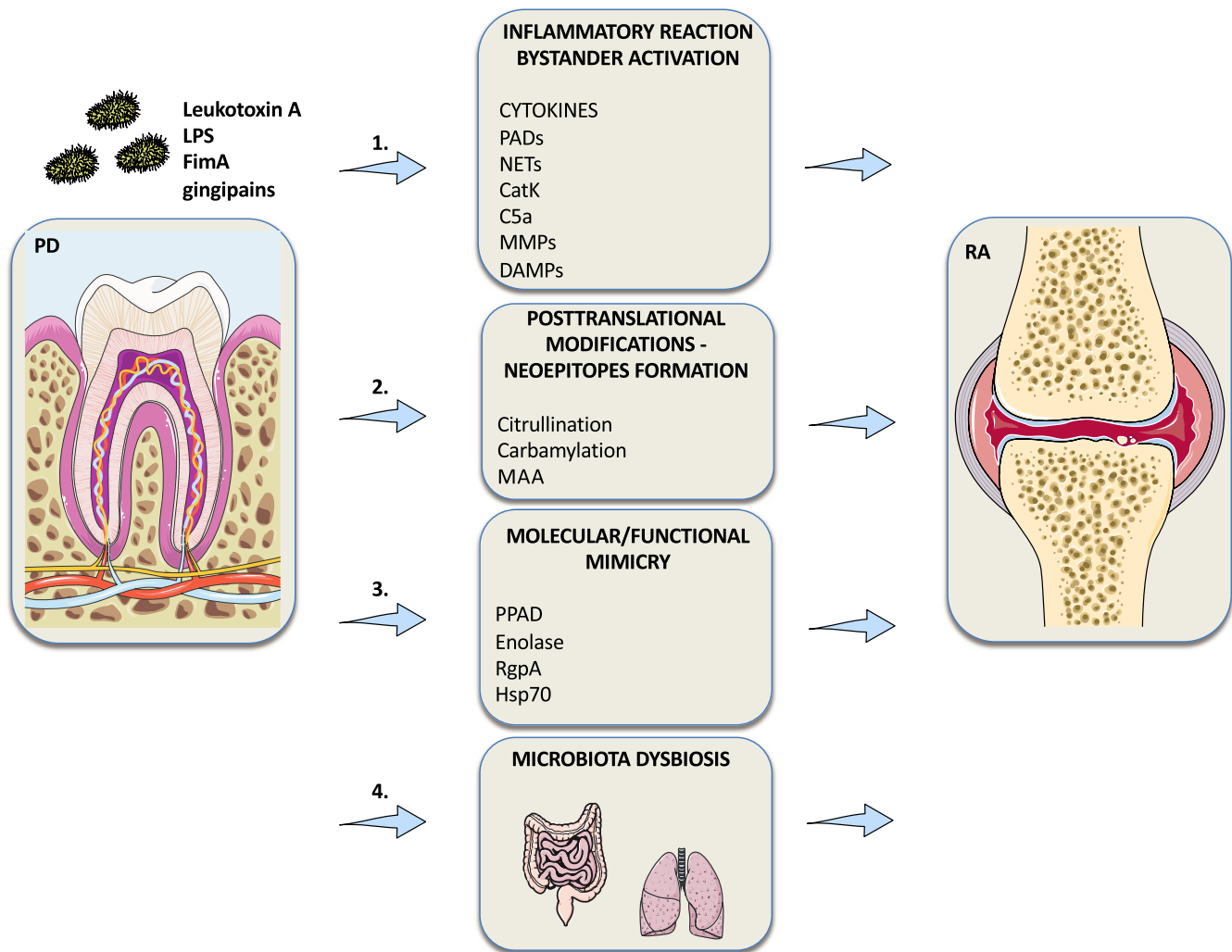
FIGURE 1 Scheme summarizing molecular pathways of rheumatoid arthritis (RA) development in the reflection of periodontal diseases (PD). (1) Periodontal pathogens induce inflammation of the periodontium by expressing a set of virulence factors, including exo and endotoxins, fimbriae, or proteolytic enzymes. The process leads to the destruction of ligament cells, activation of leukocytes, activation of enzymes, and releasing of soluble mediators, such as cytokines, components of complement system, or alarmins. Therefore, it is proposed that periodontal diseases can promote rheumatoid arthritis developed by the mechanism of bystander activation. (2) Enhanced local and systemic inflammation in periodontal diseases leads to the acceleration of posttranslational modifications. Among them are citrullination, carbamylation, and malondialdehyde-acetaldehyde adduct formation. Antibodies directed against modified proteins are found in rheumatoid arthritis patients, suggesting that the causative mechanism linking periodontal diseases with rheumatoid arthritis is based on the formation of neoepitopes. (3) Periodontal pathogens express molecules including enolase, heat shock protein 70 (Hsp70), arginine-specific gingipain (RgpA), and *Porphyromonas gingivalis*–derived peptidylarginine deiminase (PPAD), the structure and/or function of which resemble that of host proteins. This suggests that the mechanism of the molecular mimicry is underlying a causative association of periodontal diseases with rheumatoid arthritis. (4) *P. gingivalis* influences the composition of the gut and lung microbiome. The alteration of the immune system favors the development of rheumatoid arthritis. CatK, cathepsin K; DAMPs, damage-associated molecular patterns; FimA, fimbrillin; LPS, lipopolysaccharide; MAA, malondialdehyde-acetaldehyde; MMPs, matrix metalloproteinases; NETs, neutrophil extracellular traps; PADs, peptidylarginine deiminases

P. gingivalis–derived peptidylarginine deiminase among proteins expressed by *P. gingivalis*,[129] its enzyme structure and function have been extensively studied.[130-133] *P. gingivalis*–derived peptidylarginine deiminase catalyzes the same reaction as mammalian peptidylarginine deiminases; however, its activity is calcium independent, strongly favors C-terminal arginine, and also citrullinates free arginine. In protein citrullination, *P. gingivalis*–derived peptidylarginine deiminase synergizes with arginine-specific gingipains, which cleave a broad array of bacterial and host proteins to liberate C-terminal arginine residues. This cooperation is facilitated by the colocalization of *P. gingivalis*–derived peptidylarginine deiminase and arginine-specific gingipains in the outer membrane. Both enzymes working together have been shown to liberate 37 and 11 C-terminally citrullinated peptides from the two best characterized autoantigens in rheumatoid arthritis, fibrinogen and α-enolase, respectively.[130] These results contradict the earlier finding by Abdullah et al, who showed that gingipains have no influence on citrullination of yeast enolase, human vimentin, or fibrin by *P. gingivalis*–derived peptidylarginine deiminase.[134] Another controversial report published recently showed that *P. gingivalis*–derived peptidylarginine deiminase efficiently citrullinated internal arginines at the RG or RGG consensus motif in major rheumatoid arthritis autoantigens, such as fibrinogen, vimentin, hnRnP-a2/B1, and histone H1. In these experiments, recombinant *P. gingivalis*–derived peptidylarginine deiminase with the C-terminal domain was used.[135] By contrast, the mature derived peptidylarginine deiminase has no C-terminal domain, which functions as a secretory signal and is cleaved-off during translocation across the outer membrane.[129] Apparently, the presence of the C-terminal domain in the recombinant *P. gingivalis*–derived peptidylarginine deiminase facilitates autocitrullination of the *Escherichia coli*–expressed enzyme and its activity on internal arginine residues in proteins and peptides. In contrast, regarding the activity of *P. gingivalis*, fully processed *P. gingivalis*–derived peptidylarginine deiminase is practically limited to C-terminal arginine residues, and no citrullination of the native enzyme or internal arginine residues is observed.[130] Regardless of the controversy, rheumatoid arthritis patient sera screened for *P. gingivalis*–derived peptidylarginine deiminase–citrullinated epitopes uncovered 16 rheumatoid arthritis autoantigens and nine autoantigens associated with lung diseases.[135] As the anti–rheumatoid arthritis-*P. gingivalis*–derived peptidylarginine deiminase level correlated with the anti–citrullinated protein antibodies level and interstitial lung disease autoantigens, the authors proposed that treatment of *P. gingivalis* could be used as a standard routine to prevent interstitial lung disease at onset of rheumatoid arthritis.[135]

The risk of tolerance breakdown is dependent on the citrullination burden at infected periodontal sites. To this end, *P. gingivalis* effectively citrullinates its own proteins.[136] The proteomic analysis of the *P. gingivalis* citrullinome clearly indicated that secreted proteins, either released into the medium or associated with bacterial cell surface, constitute the majority of *P. gingivalis*–derived peptidylarginine deiminase–modified proteins.[137] Most of the modifications were detected at C-terminal arginine residues in accord with *P. gingivalis*–derived

peptidylarginine deiminase specificity and cooperation with arginine-gingipains. Of note, these *P. gingivalis*-derived citrullinated peptides are not recognized by anti–citrullinated protein antibodies in early signs of rheumatoid arthritis.[138] This corroborates an earlier report showing that modified peptides derived from autocitrullinated *P. gingivalis*–derived peptidylarginine deiminase are not a target for anti–citrullinated protein antibodies.[139] The conclusion from both of these studies contradicts the findings by other groups,[135,140,141] which show that citrullinated *P. gingivalis* proteins and peptides react with anti–citrullinated protein antibodies. Taken together, it is clear that the role of the *P. gingivalis* citrullinome in driving the pathology of inflammatory arthritis is still uncertain and requires more investigation.

The importance of *P. gingivalis*–derived peptidylarginine deiminase in the process of altered host epitope formation and promotion of autoimmune reactions is supported by the fact that the enzyme is heat stable and exhibits optimal activity under alkaline conditions similar to those present in the inflammatory environment.[129] Although the expression of human peptidylarginine deiminase enzymes is not affected by *P. gingivalis*–derived peptidylarginine deiminase,[142] the presence of both enzymes in the inflammatory milieu potentiates the chances of citrullination. This was verified recently by detecting *P. gingivalis*–derived peptidylarginine deiminase in synovial tissue[135] and an elevated level of antibodies to *P. gingivalis*–derived peptidylarginine deiminase in rheumatoid arthritis sera.[140]

The results of translational studies have shown that *P. gingivalis*–derived peptidylarginine deiminase expression has a profound impact on the development and progression of rheumatoid arthritis. Using a murine model of collagen induced arthritis, the authors clearly showed that disease severity was dependent on the expression of *P. gingivalis*–derived peptidylarginine deiminase.[68] This observation was confirmed using a collagen antibody–induced arthritis model.[143] Importantly, increases in the levels of autoantibodies to collagen type II and citrullinated proteins were observed only when patients were infected with bacteria expressing *P. gingivalis*–derived peptidylarginine deiminase.[68] Collectively, the data strongly support an infection-based concept of induction of anti–citrullinated protein antibodies via enzymatic mimicry, suggesting that *P. gingivalis*–derived peptidylarginine deiminase might break immune tolerance in rheumatoid arthritis.

7.1.2 | Molecular mimicry: similarity of epitopes

Antigen similarity/mimicry due to structural similarities between *P. gingivalis* antigens and self-antigens has been hypothesized to explain the role of periodontitis in rheumatoid arthritis development. Among the specific antigens identified in rheumatoid arthritis patients is citrullinated enolase.[144] Lundberg et al[145] proposed that this protein plays a central role in the initiation of the periodontal diseases–induced pathogenic pathway that leads to rheumatoid arthritis development. The aforementioned hypothesis comes from studies showing that citrullinated α-enolase peptide 1, which is detected in the majority of rheumatoid arthritis patients (up to 62%),

shows 82% sequence homology with *P. gingivalis*–derived enolase. Moreover, it was demonstrated that the antibody levels to citrullinated α-enolase peptide 1 correlated with those to the bacterial peptide and that they cross-react with citrullinated recombinant *P. gingivalis* enolase.[146] Translational studies using DR4-IE transgenic mice supported the role of the bacterial enolase. Kinloch et al showed that bacterial enolase induces arthritis as efficiently as the human enolase does and induced the humoral response by producing antibodies to both citrullinated and unmodified human enolase.[147] Based on these results, the unmodified form of *P. gingivalis* α-enolase may be important in initiating the corresponding subset of human rheumatoid arthritis. Further, these data provide a strong basis for the causative association between rheumatoid arthritis and periodontal diseases, based on the molecular mimicry hypothesis.

Heat shock protein 60 is considered to be another bacterial-derived molecule that is highly cross-reactive with human antibodies. The analysis of heat shock protein 60 sequences from different pathogens, including *Chlamydia pneumoniae* and *Mycobacterium tuberculosis*, revealed that only the *P. gingivalis* epitope identified within this chaperone sequence is predominantly and frequently recognized by antibodies in the serum of rheumatoid arthritis patients.[148] The heat shock protein 60 sequence is highly conserved; therefore, it cannot be excluded that other types of bacteria could contribute to molecular mimicry and the breakdown of immune tolerance in inflamed gingival mucosae.

Antigenic determinants of type II collagen play a role in collagen-induced arthritis.[149] Recently, Peng et al identified an amino acid sequence similar to the arginine-specific gingipain A catalytic domain and rat type II collagen, suggesting a potentially immunogenic role of gingipain. The authors demonstrated that preimmunization of rats with purified recombinant arginine-specific gingipain A triggered a potent protective immune response that manifested as an increase in the level of type II collagen–specific antibodies that, in turn, alleviated arthritis in the joints of the animals.[150] This evidence suggests that translocation of *P. gingivalis*–expressing arginine-specific gingipain A in the synovium may exacerbate the inflammatory response that promotes rheumatoid arthritis. Therefore, it may be beneficial to preimmunize or apply small-molecule inhibitors of gingipain (Figure 1).

8 | SUMMARY

Consistent with the hypothesis for the mucosal origins of rheumatoid arthritis development, it is clear that periodontal pathogens should be considered as important environmental players contributing to immune abnormality in rheumatoid arthritis patients. The detailed analysis of rheumatoid arthritis etiology in this review suggests that dysbiosis, responsible for chronic inflammation of the periodontium or in the gut, could trigger autoimmunity via several mechanisms, including bystander activation, amplification of autoimmunity by cytokines, epitope spreading, autoantigen overproduction, microbial translocation, and molecular mimicry. Considering that development

of microbial dysbiosis in the gut and the oral cavity is preventable suggests that appropriate diet, the use of probiotics, and strict oral hygiene should at least slow down the development of rheumatoid arthritis and lessen the disease severity.

REFERENCES

1. Maixner F, Thomma A, Cipollini G, Widder S, Rattei T, Zink A. Metagenomic analysis reveals presence of *Treponema denticola* in a tissue biopsy of the Iceman. *PLoS One*. 2014;9:e99994.
2. Adler CJ, Dobney K, Weyrich LS, et al. Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and industrial revolutions. *Nat Genet*. 2013;45:450-455.
3. Warinner C, Rodrigues JFM, Vyas R, et al. Pathogens and host immunity in the ancient human oral cavity. *Nat Genet*. 2014;46:336-344.
4. Gonçalves PCG, Griffiths G, Rawlinson A. A study of the periodontal state of a late Medieval United Kingdom population. *Arch Oral Biol*. 2015;60:1797-1801.
5. Raitapuro-Murray T, Molleson TI, Hughes FJ. The prevalence of periodontal disease in a Romano-British population c. 200-400 AD. *Br Dent J*. 2014;217:459-466.
6. Philips A, Stolarek I, Handschuh L, et al. Analysis of oral microbiome from fossil human remains revealed the significant differences in virulence factors of modern and ancient *Tannerella forsythia*. *BMC Genom*. 2020;21:402.
7. Landré-Beauvais AJ. The first description of rheumatoid arthritis. Unabridged text of the doctoral dissertation presented in 1800. *Joint Bone Spine*. 2001;68:130-143.
8. Potempa J, Mydel P, Koziel J. The case for periodontitis in the pathogenesis of rheumatoid arthritis. *Nat Rev Rheumatol*. 2017;13:606-620.
9. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum*. 1987;30:1205-1213.
10. Raychaudhuri S, Sandor C, Stahl EA, et al. Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. *Nat Genet*. 2012;44:291-296.
11. Gehlot P, Volk SL, Rios HF, Jepsen KJ, Holoshitz J. Spontaneous destructive periodontitis and skeletal bone damage in transgenic mice carrying a human shared epitope-coding HLA-DRB1 allele. *RMD Open*. 2016;2:e000349.
12. Sandal I, Karydis A, Luo J, et al. Bone loss and aggravated autoimmune arthritis in HLA-DRβ1-bearing humanized mice following oral challenge with *Porphyromonas gingivalis*. *Arthritis Res Ther*. 2016;18:249.
13. Schulz S, Zimmer P, Pütz N, Jurianz E, Schaller H-G, Reichert S. rs2476601 in *PTPN22* gene in rheumatoid arthritis and periodontitis—a possible interface? *J Transl Med*. 2020;18:389.
14. Kobayashi T, Kido J-I, Ishihara Y, et al. The *KCNQ1* gene polymorphism as a shared genetic risk for rheumatoid arthritis and chronic periodontitis in Japanese adults: a pilot case-control study. *J Periodontol*. 2018;89:315-324.

15. Shi L-X, Zhang L, Zhang D-L, et al. Association between TNF-α G-308A (rs1800629) polymorphism and susceptibility to chronic periodontitis and type 2 diabetes mellitus: a meta-analysis. *J Periodontal Res.* 2021;56:226-235.

16. Di Giuseppe D, Discacciati A, Orsini N, Wolk A. Cigarette smoking and risk of rheumatoid arthritis: a dose-response meta-analysis. *Arthritis Res Ther.* 2014;16:r61.

17. Sato K, Takahashi N, Kato T, et al. Aggravation of collagen-induced arthritis by orally administered *Porphyromonas gingivalis* through modulation of the gut microbiota and gut immune system. *Sci Rep.* 2017;7:6955.

18. Flak MB, Colas RA, Muñoz-Atienza E, Curtis MA, Dalli J, Pitzalis C. Inflammatory arthritis disrupts gut resolution mechanisms, promoting barrier breakdown by *Porphyromonas gingivalis. JCI Insight.* 2019;4:e125191.

19. Bennike TB, Ellingsen T, Glerup H, et al. Proteome analysis of rheumatoid arthritis gut mucosa. *J Proteome Res.* 2017;16:346-354.

20. Kobayashi T, Yoshie H. Host responses in the link between periodontitis and rheumatoid arthritis. *Curr Oral Health Rep.* 2015;2:1-8.

21. Delima AJ, Van Dyke TE. Origin and function of the cellular components in gingival crevice fluid. *Periodontology.* 2000;2003(31):55-76.

22. Vitkov L, Klappacher M, Hannig M, Krautgartner WD. Extracellular neutrophil traps in periodontitis. *J Periodontal Res.* 2009;44:664-672.

23. Woolley DE, Crossley MJ, Evanson JM. Collagenase at sites of cartilage erosion in the rheumatoid joint. *Arthritis Rheum.* 1977;20:1231-1239.

24. Crotti T, Smith MD, Hirsch R, et al. Receptor activator NF κB ligand (RANKL) and osteoprotegerin (OPG) protein expression in periodontitis. *J Periodontal Res.* 2003;38:380-387.

25. Haynes DR, Crotti TN, Loric M, Bain GI, Atkins GJ, Findlay DM. Osteoprotegerin and receptor activator of nuclear factor kappaB ligand (RANKL) regulate osteoclast formation by cells in the human rheumatoid arthritic joint. *Rheumatology (Oxford, England).* 2001;40:623-630.

26. Dutzan N, Kajikawa T, Abusleme L, et al. A dysbiotic microbiome triggers T$_H$17 cells to mediate oral mucosal immunopathology in mice and humans. *Sci Transl Med.* 2018;10:eaat0797.

27. van Hamburg JP, Tas SW. Molecular mechanisms underpinning T helper 17 cell heterogeneity and functions in rheumatoid arthritis. *J Autoimmun.* 2018;87:69-81.

28. Shimada Y, Komatsu Y, Ikezawa-Suzuki I, Tai H, Sugita N, Yoshie H. The effect of periodontal treatment on serum leptin, interleukin-6, and C-reactive protein. *J Periodontol.* 2010;81:1118-1123.

29. Schmickler J, Rupprecht A, Patschan S, et al. Cross-sectional evaluation of periodontal status and microbiologic and rheumatoid parameters in a large cohort of patients with rheumatoid arthritis. *J Periodontol.* 2017;88:368-379.

30. Eriksson K, Fei G, Lundmark A, et al. Periodontal health and oral microbiota in patients with rheumatoid arthritis. *J Clin Med.* 2019;8:630.

31. Joseph R, Rajappan S, Nath SG, Paul BJ. Association between chronic periodontitis and rheumatoid arthritis: a hospital-based case-control study. *Rheumatol Int.* 2013;33:103-109.

32. Hashimoto M, Yamazaki T, Hamaguchi M, et al. Periodontitis and *Porphyromonas gingivalis* in preclinical stage of arthritis patients. *PLoS One.* 2015;10:e0122121.

33. Bello-Gualtero JM, Lafaurie GI, Hoyos LX, et al. Periodontal disease in individuals with a genetic risk of developing arthritis and early rheumatoid arthritis: a cross-sectional study. *J Periodontol.* 2016;87:346-356.

34. Terao C, Asai K, Hashimoto M, et al. Significant association of periodontal disease with anti-citrullinated peptide antibody in a Japanese healthy population—the Nagahama study. *J Autoimmun.* 2015;59:85-90.

35. Unriza-Puin S, Bautista-Molano W, Lafaurie GI, et al. Are obesity, ACPAs and periodontitis conditions that influence the risk of developing rheumatoid arthritis in first-degree relatives? *Clin Rheumatol.* 2017;36:799-806.

36. Loutan L, Alpizar-Rodriguez D, Courvoisier DS, Finckh A, Mombelli A, Giannopoulou C. Periodontal status correlates with anti-citrullinated protein antibodies in first-degree relatives of individuals with rheumatoid arthritis. *J Clin Periodontol.* 2019;46:690-698.

37. Ortiz P, Bissada NF, Palomo L, et al. Periodontal therapy reduces the severity of active rheumatoid arthritis in patients treated with or without tumor necrosis factor inhibitors. *J Periodontol.* 2009;80:535-540.

38. Al-Katma MK, Bissada NF, Bordeaux JM, Sue J, Askari AD. Control of periodontal infection reduces the severity of active rheumatoid arthritis. *J Clin Rheumatol.* 2007;13:134-137.

39. Gargiulo AVJ, Robinson J, Toto PD, Gargiulo AW. Identification of rheumatoid factor in periodontal disease. *J Periodontol.* 1982;53:568-577.

40. Lappin DF, Apatzidou D, Quirke A-M, et al. Influence of periodontal disease, *Porphyromonas gingivalis* and cigarette smoking on systemic anti-citrullinated peptide antibody titres. *J Clin Periodontol.* 2013;40:907-915.

41. Qiao Y, Wang Z, Li Y, Han Y, Zhou Y, Cao X. Rheumatoid arthritis risk in periodontitis patients: a systematic review and meta-analysis. *Joint Bone Spine.* 2020;87:556-564.

42. Okada M, Kobayashi T, Ito S, et al. Antibody responses to periodontopathic bacteria in relation to rheumatoid arthritis in Japanese adults. *J Periodontol.* 2011;82:1433-1441.

43. Mikuls TR, Thiele GM, Deane KD, et al. *Porphyromonas gingivalis* and disease-related autoantibodies in individuals at increased risk of rheumatoid arthritis. *Arthritis Rheum.* 2012;64:3522-3530.

44. Hitchon CA, Chandad F, Ferucci ED, et al. Antibodies to *Porphyromonas gingivalis* are associated with anticitrullinated protein antibodies in patients with rheumatoid arthritis and their relatives. *J Rheumatol.* 2010;37:1105-1112.

45. Arvikar SL, Collier DS, Fisher MC, et al. Clinical correlations with *Porphyromonas gingivalis* antibody responses in patients with early rheumatoid arthritis. *Arthritis Res Ther.* 2013;15:R109.

46. Seror R, Le Gall-David S, Bonnaure-Mallet M, et al. Association of anti–*Porphyromonas gingivalis* antibody titers with nonsmoking status in early rheumatoid arthritis: results from the prospective French cohort of patients with early rheumatoid arthritis. *Arthritis Rheumatol (Hoboken, NJ).* 2015;67:1729-1737.

47. Ogrendik M, Kokino S, Ozdemir F, Bird PS, Hamlet S. Serum antibodies to oral anaerobic bacteria in patients with rheumatoid arthritis. *MedGenMed.* 2005;7:2.

48. Johansson L, Sherina N, Kharlamova N, et al. Concentration of antibodies against *Porphyromonas gingivalis* is increased before the onset of symptoms of rheumatoid arthritis. *Arthritis Res Ther.* 2016;18:201.

49. Mankia K, Cheng Z, Do T, et al. Prevalence of periodontal disease and periodontopathic bacteria in anti–cyclic citrullinated protein antibody–positive at-risk adults without arthritis. *JAMA Netw Open.* 2019;2:e195394.

50. Kim J-H, Choi IA, Lee JY, et al. Periodontal pathogens and the association between periodontitis and rheumatoid arthritis in Korean adults. *J Periodontal Implant Sci.* 2018;48:347-359.

51. Bae S-C, Lee YH. Association between anti–*Porphyromonas gingivalis* antibody, anti–citrullinated protein antibodies, and rheumatoid arthritis: a meta-analysis. *Z Rheumatol.* 2018;77:522-532.

52. Ceccarelli F, Orrù G, Pilloni A, et al. *Porphyromonas gingivalis* in the tongue biofilm is associated with clinical outcome in rheumatoid arthritis patients. *Clin Exp Immunol.* 2018;194:244-252.

53. Erciyas K, Sezer U, Üstün K, et al. Effects of periodontal therapy on disease activity and systemic inflammation in rheumatoid arthritis patients. *Oral Dis.* 2013;19:394-400.

54. Ribeiro J, Leão A, Novaes AB. Periodontal infection as a possible severity factor for rheumatoid arthritis. *J Clin Periodontol.* 2005;32:412-416.

55. Monsarrat P, Fernandez de Grado G, Constantin A, et al. The effect of periodontal treatment on patients with rheumatoid arthritis: the ESPERA randomised controlled trial. *Joint Bone Spine.* 2019;86:600-609.

56. Cosgarea R, Tristiu R, Dumitru RB, et al. Effects of non-surgical periodontal therapy on periodontal laboratory and clinical data as well as on disease activity in patients with rheumatoid arthritis. *Clin Oral Invest.* 2019;23:141-151.

57. Ogrendik M. Rheumatoid arthritis is linked to oral bacteria: etiological association. *Mod Rheumatol.* 2009;19:453-456.

58. Niu X, Chen G. Clinical biomarkers and pathogenic-related cytokines in rheumatoid arthritis. *J Immunol Res.* 2014;2014:698192.

59. Mayer Y, Balbir-Gurman A, Machtei EE. Anti–tumor necrosis factor-alpha therapy and periodontal parameters in patients with rheumatoid arthritis. *J Periodontol.* 2009;80:1414-1420.

60. Rinaudo-Gaujous M, Blasco-Baque V, Miossec P, et al. Infliximab induced a dissociated response of severe periodontal biomarkers in rheumatoid arthritis patients. *J Clin Med.* 2019;8:751.

61. Kobayashi T, Ito S, Kobayashi D, et al. Interleukin-6 receptor inhibitor tocilizumab ameliorates periodontal inflammation in patients with rheumatoid arthritis and periodontitis as well as tumor necrosis factor inhibitors. *Clin Exp Dental Res.* 2015;1:63-73.

62. Ancuța C, Chirieac R, Ancuța E, et al. Exploring the role of interleukin-6 receptor inhibitor tocilizumab in patients with active rheumatoid arthritis and periodontal disease. *J Clin Med.* 2021;10:878.

63. Äyräväinen L, Leirisalo-Repo M, Kuuliala A, et al. Periodontitis in early and chronic rheumatoid arthritis: a prospective follow-up study in Finnish population. *BMJ Open.* 2017;7:e011916.

64. Jung G-U, Han J-Y, Hwang K-G, Park C-J, Stathopoulou PG, Fiorellini JP. Effects of conventional synthetic disease-modifying antirheumatic drugs on response to periodontal treatment in patients with rheumatoid arthritis. *Biomed Res Int.* 2018;2018:1465402.

65. Cantley MD, Haynes DR, Marino V, Bartold PM. Pre-existing periodontitis exacerbates experimental arthritis in a mouse model. *J Clin Periodontol.* 2011;38:532-541.

66. Bartold PM, Marino V, Cantley M, Haynes DR. Effect of *Porphyromonas gingivalis*–induced inflammation on the development of rheumatoid arthritis. *J Clin Periodontol.* 2010;37:405-411.

67. Marchesan JT, Gerow EA, Schaff R, et al. *Porphyromonas gingivalis* oral infection exacerbates the development and severity of collagen-induced arthritis. *Arthritis Res Ther.* 2013;15:R186.

68. Maresz KJ, Hellvard A, Sroka A, et al. *Porphyromonas gingivalis* facilitates the development and progression of destructive arthritis through its unique bacterial peptidylarginine deiminase (PAD). *PLoS Pathog.* 2013;9:e1003627.

69. Sakaguchi W, To M, Yamamoto Y, et al. Detection of anti–citrullinated protein antibody (ACPA) in saliva for rheumatoid arthritis using DBA mice infected with *Porphyromonas gingivalis. Arch Oral Biol.* 2019;108:104510.

70. de Aquino SG, Abdollahi-Roodsaz S, Koenders MI, et al. Periodontal pathogens directly promote autoimmune experimental arthritis by inducing a TLR2 and IL-1-driven Th17 response. *J Immunol (Baltimore, Md: 1950).* 2014;192:4103-4111.

71. Ebbers M, Lübcke PM, Volzke J. Interplay between *P. gingivalis*, *F. nucleatum* and *A. actinomycetemcomitans* in murine alveolar bone loss, arthritis onset and progression. *Sci Rep.* 2018;8:15129.

72. Chukkapalli S, Rivera-Kweh M, Gehlot P, et al. Periodontal bacterial colonization in synovial tissues exacerbates collagen-induced arthritis in B10.RIII mice. *Arthritis Res Ther.* 2016;18:161.

73. Lübcke PM, Ebbers MNB, Volzke J, et al. Periodontal treatment prevents arthritis in mice and methotrexate ameliorates periodontal bone loss. *Sci Rep.* 2019;9:8128.

74. Yamakawa M, Ouhara K, Kajiya M, et al. Porphyromonas gingivalis infection exacerbates the onset of rheumatoid arthritis in SKG mice. *Clin Exp Immunol.* 2016;186:177-189.

75. Courbon G, Rinaudo-Gaujous M, Blasco-Baque V, et al. *Porphyromonas gingivalis* experimentally induces periodontis and an anti-CCP2-associated arthritis in the rat. *Ann Rheum Dis.* 2019;78:594-599.

76. Ramamurthy NS, Greenwald RA, Celiker MY, Shi EY. Experimental arthritis in rats induces biomarkers of periodontitis which are ameliorated by gene therapy with tissue inhibitor of matrix metalloproteinases. *J Periodontol.* 2005;76:229-233.

77. Trombone AP, Claudino M, Colavite P, et al. Periodontitis and arthritis interaction in mice involves a shared hyper-inflammatory genotype and functional immunological interferences. *Genes Immun.* 2010;11:479-489.

78. Queiroz-Junior CM, Madeira MFM, Coelho FM, et al. Experimental arthritis triggers periodontal disease in mice: involvement of TNF-α and the oral microbiota. *J Immunol (Baltimore, Md: 1950).* 2011;187:3821-3830.

79. Martins P, Fonseca JE. How to investigate: pre-clinical rheumatoid arthritis. *Best Pract Res Clin Rheumatol.* 2019;33:101438.

80. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Investig.* 1998;101:273-281.

81. Nesse W, Westra J, van der Wal JE, et al. The periodontium of periodontitis patients contains citrullinated proteins which may play a role in ACPA (anti–citrullinated protein antibody) formation. *J Clin Periodontol.* 2012;39:599-607.

82. Lee YH, Baharuddin NA, Chan SW, et al. Localisation of citrullinated and carbamylated proteins in inflamed gingival tissues from rheumatoid arthritis patients. *Clin Oral Invest.* 2021;25:1441-1450.

83. Pratesi F, Dioni I, Tommasi C, et al. Antibodies from patients with rheumatoid arthritis target citrullinated histone 4 contained in neutrophils extracellular traps. *Ann Rheum Dis.* 2014;73:1414-1422.

84. Corsiero E, Bombardieri M, Carlotti E, et al. Single cell cloning and recombinant monoclonal antibodies generation from RA synovial B cells reveal frequent targeting of citrullinated histones of NETs. *Ann Rheum Dis.* 2016;75:1866-1875.

85. Janssen KMJ, de Smit MJ, Withaar C, et al. Autoantibodies against citrullinated histone H3 in rheumatoid arthritis and periodontitis patients. *J Clin Periodontol.* 2017;44:577-584.

86. Wang Z, Nicholls SJ, Rodriguez ER, et al. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. *Nat Med.* 2007;13:1176-1184.

87. Shi J, Knevel R, Suwannalai P, et al. Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid arthritis and predict joint damage. *Proc Natl Acad Sci USA.* 2011;108:17372-17377.

88. Li L, Deng C, Chen S, et al. Meta-analysis: diagnostic accuracy of anti–carbamylated protein antibody for rheumatoid arthritis. *PLoS One.* 2016;11:e0159000.

89. Stoop JN, Liu B-S, Shi J, et al. Antibodies specific for carbamylated proteins precede the onset of clinical symptoms in mice with collagen induced arthritis. *PLoS One.* 2014;9:e102163.

90. Verheul MK, Vierboom MPM, 't Hart BA, Toes REM, Trouw LA. Anti–carbamylated protein antibodies precede disease onset in monkeys with collagen-induced arthritis. *Arthritis Res Ther.* 2017;19:246.

91. Thiele GM, Duryee MJ, Anderson DR, et al. Malondialdehyde-acetaldehyde adducts and anti–malondialdehyde-acetaldehyde antibodies in rheumatoid arthritis. *Arthritis Rheumatol (Hoboken, NJ).* 2015;67:645-655.

92. Mikuls TR, Edison J, Meeshaw E, et al. Autoantibodies to malondialdehyde-acetaldehyde are detected prior to rheumatoid

arthritis diagnosis and after other disease specific autoantibodies. *Arthritis Rheumatol (Hoboken, NJ).* 2020;72:2025-2029.

93. Bright R, Thiele GM, Manavis J, Mikuls TR, Payne JB, Bartold PM. Gingival tissue, an extrasynovial source of malondialdehyde-acetaldehyde adducts, citrullinated and carbamylated proteins. *J Periodontal Res.* 2018;53:139-143.

94. Sturfelt G, Truedsson L. Complement in the immunopathogenesis of rheumatic disease. *Nat Rev Rheumatol.* 2012;8:458-468.

95. Jose PJ, Moss IK, Maini RN, Williams TJ. Measurement of the chemotactic complement fragment C5a in rheumatoid synovial fluids by radioimmunoassay: role of C5a in the acute inflammatory phase. *Ann Rheum Dis.* 1990;49:747-752.

96. Grant EP, Picarella D, Burwell T, et al. Essential role for the C5a receptor in regulating the effector phase of synovial infiltration and joint destruction in experimental arthritis. *J Exp Med.* 2002;196:1461-1471.

97. Andersson C, Wenander CS, Usher PA, et al. Rapid-onset clinical and mechanistic effects of anti-C5aR treatment in the mouse collagen-induced arthritis model. *Clin Exp Immunol.* 2014;177:219-233.

98. Abe T, Hosur KB, Hajishengallis E, et al. Local complement-targeted intervention in periodontitis: proof-of-concept using a C5a receptor (CD88) antagonist. *J Immunol (Baltimore, Md: 1950).* 2012;189(11):5442–5448.

99. Wingrove JA, DiScipio RG, Chen Z, Potempa J, Travis J, Hugli TE. Activation of complement components C3 and C5 by a cysteine proteinase (gingipain-1) from *Porphyromonas* (Bacteroides) *gingivalis. J Biol Chem.* 1992;267:18902-18907.

100. Munenaga S, Ouhara K, Hamamoto Y, et al. The involvement of C5a in the progression of experimental arthritis with *Porphyromonas gingivalis* infection in SKG mice. *Arthritis Res Ther.* 2018;20:247.

101. 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.

102. Maijer KI, Gudmann NS, Karsdal MA, Gerlag DM, Tak PP, Bay-Jensen AC. Neo-epitopes—fragments of cartilage and connective tissue degradation in early rheumatoid arthritis and unclassified arthritis. *PLoS One.* 2016;11:e0149329.

103. Äyräväinen L, Heikkinen AM, Kuuliala A, et al. Inflammatory biomarkers in saliva and serum of patients with rheumatoid arthritis with respect to periodontal status. *Ann Med.* 2018;50:333-344.

104. Guentsch A, Hirsch C, Pfister W, et al. Cleavage of IgG1 in gingival crevicular fluid is associated with the presence of *Porphyromonas gingivalis. J Periodontal Res.* 2013;48:458-465.

105. Ebe N, Hara-Yokoyama M, Iwasaki K, et al. Pocket epithelium in the pathological setting for HMGB1 release. *J Dent Res.* 2011;90:235-240.

106. Bui FQ, Johnson L, Roberts J, et al. *Fusobacterium nucleatum* infection of gingival epithelial cells leads to NLRP3 inflammasome–dependent secretion of IL-1β and the danger signals ASC and HMGB1. *Cell Microbiol.* 2016;18:970-981.

107. Jun H-K, Jung Y-J, Choi B-K *Treponema denticola, Porphyromonas gingivalis,* and *Tannerella forsythia* induce cell death and release of endogenous danger signals. *Arch Oral Biol.* 2017;73:72-78.

108. Yoshihara-Hirata C, Yamashiro K, Yamamoto T, et al. Anti–HMGB1 neutralizing antibody attenuates periodontal inflammation and bone resorption in a murine periodontitis model. *Infect Immun.* 2018;86:111-118.

109. Wang S, Wang Y. Peptidylarginine deiminases in citrullination, gene regulation, health and pathogenesis. *Biochem Biophys Acta.* 2013;1829:1126-1135.

110. Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. *J Exp Med.* 2010;207:1853-1862.

111. Kilsgård O, Andersson P, Malmsten M, et al. Peptidylarginine deiminases present in the airways during tobacco smoking

and inflammation can citrullinate the host defense peptide LL-37, resulting in altered activities. *Am J Respir Cell Mol Biol.* 2012;46:240-248.

112. Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, et al. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Sci Transl Med.* 2013;5:178ra40.

113. Wright HL, Moots RJ, Edwards SW. The multifactorial role of neutrophils in rheumatoid arthritis. *Nat Rev Rheumatol.* 2014;10:593-601.

114. Scott DA, Krauss J. Neutrophils in periodontal inflammation. *Front Oral Biol.* 2012;15:56-83.

115. Vitkov L, Klappacher M, Hannig M, Krautgartner WD. Neutrophil fate in gingival crevicular fluid. *Ultrastruct Pathol.* 2010;34:25-30.

116. Delbosc S, Alsac J-M, Journe C, et al. Participates in pathogenesis of human abdominal aortic aneurysm by neutrophil activation. Proof of concept in rats. *PLoS One.* 2011;6(4):e18679.

117. Bryzek D, Ciaston I, Dobosz E, et al. Triggering NETosis via protease-activated receptor (PAR)-2 signaling as a mechanism of hijacking neutrophils function for pathogen benefits. *PLoS Pathog.* 2019;15:e1007773.

118. Konig MF, Abusleme L, Reinholdt J, et al. *Aggregatibacter actinomycetemcomitans*–induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. *Sci Transl Med.* 2016;8:369ra176.

119. Engström M, Eriksson K, Lee L, et al. Increased citrullination and expression of peptidylarginine deiminases independently of *P. gingivalis* and *A. actinomycetemcomitans* in gingival tissue of patients with periodontitis. *J Transl Med.* 2018;16:214.

120. Scher JU, Sczesnak A, Longman RS, et al. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *eLife.* 2013;2:e01202.

121. Maeda Y, Kurakawa T, Umemoto E, et al. Dysbiosis contributes to arthritis development via activation of autoreactive T cells in the intestine. *Arthritis Rheumatol (Hoboken, NJ).* 2016;68:2646-2661.

122. Pianta A, Arvikar S, Strle K, et al. Evidence of the immune relevance of *Prevotella copri*, a gut microbe, in patients with rheumatoid arthritis. *Arthritis Rheumatol (Hoboken, NJ).* 2017;69:964-975.

123. Pianta A, Chiumento G, Ramsden K, et al. Identification of novel, immunogenic HLA-DR-presented *Prevotella copri* peptides in patients with rheumatoid arthritis: patients with rheumatoid arthritis. *Arthritis Rheumatol (Hoboken, NJ).* 2021;37:2200-2205. doi:10.1002/art.41807. published online May.

124. Alpizar-Rodriguez D, Lesker TR, Gronow A, et al. *Prevotella copri* in individuals at risk for rheumatoid arthritis. *Ann Rheum Dis.* 2019;78:590-593.

125. Kishikawa T, Maeda Y, Nii T, et al. Metagenome-wide association study of gut microbiome revealed novel aetiology of rheumatoid arthritis in the Japanese population. *Ann Rheum Dis.* 2020;79:103-111.

126. Kishikawa T, Maeda Y, Nii T. The positive correlation between *Porphyromonas gingivalis* and *Prevotella* spp. Response to: 'Comment on "Metagenome-wide association study of gut microbiome revealed novel aetiology of rheumatoid arthritis in the Japanese population" by Kishikawa et al.' by Kitamura et al. *Ann Rheum Dis.* 2020; published online May. doi:10.1136/annrheumdis-2020-217897. Online ahead of print

127. Arimatsu K, Yamada H, Miyazawa H, et al. Oral pathobiont induces systemic inflammation and metabolic changes associated with alteration of gut microbiota. *Sci Rep.* 2014;4:4828.

128. Nakajima M, Arimatsu K, Kato T, et al. Oral administration of *P. gingivalis* induces dysbiosis of gut microbiota and impaired barrier function leading to dissemination of enterobacteria to the liver. *PLoS One.* 2015;10(7):e0134234.

129. McGraw WT, Potempa J, Farley D, Travis J. Purification, characterization, and sequence analysis of a potential virulence factor

from *Porphyromonas gingivalis*, peptidylarginine deiminase. *Infect Immun.* 1999;67:3248-3256.

130. Montgomery AB, Kopec J, Shrestha L, et al. Crystal structure of *Porphyromonas gingivalis* peptidylarginine deiminase: implications for autoimmunity in rheumatoid arthritis. *Ann Rheum Dis.* 2016;75:1255-1261.

131. Koziel J, Mydel P, Potempa J. The link between periodontal disease and rheumatoid arthritis. *Curr Rheumatol Rep.* 2014;16:408.

132. Goulas T, Mizgalska D, Garcia-Ferrer I, et al. Structure and mechanism of a bacterial host–protein citrullinating virulence factor, *Porphyromonas gingivalis* peptidylarginine deiminase. *Sci Rep.* 2015;5:11969.

133. Bereta G, Goulas T, Madej M, et al. Structure, function, and inhibition of a genomic/clinical variant of *Porphyromonas gingivalis* peptidylarginine deiminase. *Protein Sci.* 2019;28:478-486.

134. Abdullah S-N, Farmer E-A, Spargo L, Logan R, Gully N *Porphyromonas gingivalis* peptidylarginine deiminase substrate specificity. *Anaerobe.* 2013;23:102-108.

135. Jenning M, Marklein B, Ytterberg J, et al. Bacterial citrullinated epitopes generated by *Porphyromonas gingivalis* infection—a missing link for ACPA production. *Ann Rheum Dis.* 2020;79:1194-1202.

136. Wegner N, Wait R, Sroka A, et al. Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and α-enolase: implications for autoimmunity in rheumatoid arthritis. *Arthritis Rheum.* 2010;62:2662-2672.

137. Stobernack T, Glasner C, Junker S, et al. Extracellular proteome and citrullinome of the oral pathogen *Porphyromonas gingivalis*. *J Proteome Res.* 2016;15:4532-4543.

138. Muñoz-Atienza E, Flak MB, Sirr J, et al. The *P gingivalis* autocitrullinome is not a target for ACPA in early rheumatoid arthritis. *J Dent Res.* 2020;99:456-462.

139. Konig MF, Paracha AS, Moni M, Bingham CO 3rd, Andrade F. Defining the role of *Porphyromonas gingivalis* peptidylarginine deiminase (PPAD) in rheumatoid arthritis through the study of PPAD biology. *Ann Rheum Dis.* 2015;74:2054-2061.

140. Quirke A-M, Lugli EB, Wegner N, et al. Heightened immune response to autocitrullinated *Porphyromonas gingivalis* peptidylarginine deiminase: a potential mechanism for breaching immunologic tolerance in rheumatoid arthritis. *Ann Rheum Dis.* 2014;73:263-269.

141. Quirke A-M, Lundberg K, Potempa J, Mikuls J, Venables J. PPAD remains a credible candidate for inducing autoimmunity in rheumatoid arthritis: comment on the article by Konig et al. *Ann Rheum Dis.* 2015;74:e7.

142. Marchant C, Smith MD, Proudman S, Haynes DR, Bartold PM. Effect of *Porphyromonas gingivalis* on citrullination of proteins by macrophages in vitro. *J Periodontol.* 2013;84:1272-1280.

143. Gully N, Bright R, Marino V, et al. *Porphyromonas gingivalis* peptidylarginine deiminase, a key contributor in the pathogenesis of experimental periodontal disease and experimental arthritis. *PLoS One.* 2014;9:e100838.

144. Tilvawala R, Nguyen SH, Maurais AJ, et al. The rheumatoid arthritis–associated citrullinome. *Cell Chem Biol.* 2018;25:691-704.e6.

145. Lundberg K, Wegner N, Yucel-Lindberg T, Venables PJ. Periodontitis in RA—the citrullinated enolase connection. *Nat Rev Rheumatol.* 2010;6:727-730.

146. Lundberg K, Kinloch A, Fisher BA, et al. Antibodies to citrullinated α-enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase. *Arthritis Rheum.* 2008;58:3009-3019.

147. Kinloch AJ, Alzabin S, Brintnell W, et al. Immunization with *Porphyromonas gingivalis* enolase induces autoimmunity to mammalian α-enolase and arthritis in DR4-IE-transgenic mice. *Arthritis Rheum.* 2011;63:3818-3823.

148. Jeong E, Lee J-Y, Kim S-J, Choi J. Predominant immunoreactivity of *Porphyromonas gingivalis* heat shock protein in autoimmune diseases. *J Periodontal Res.* 2012;47:811-816.

149. Trentham DE, Townes AS, Kang AH, David JR. Humoral and cellular sensitivity to collagen in type II collagen-induced arthritis in rats. *J Clin Investig.* 1978;61:89-96.

150. Peng H-Y, Chen S-Y, Siao S-H, et al. Targeting a cysteine protease from a pathobiont alleviates experimental arthritis. *Arthritis Res Ther.* 2020;22:114.