Gums and joints: is there a connection? Part two: the biological link

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Key points

- Discusses the most plausible theories to explain the biological link between RA and PD.
- Outlines the common risk factors between the two diseases.
- Proposes new studies to prove or disprove a causal relationship between PD and RA.

Abstract

Rheumatoid arthritis (RA) and periodontitis (PD) are inflammatory diseases characterised by an exacerbated immune-inflammatory reaction that leads to the destruction of bone and other connective tissues that share numerous similarities. Although a significant and independent association between these two conditions has been described, the pathophysiological processes that may explain this relationship remain unknown and multiple theories have been proposed. This review presents the most important theories currently proposed to explain the biological link between RA and PD.

Introduction

The available evidence to date suggests a significant and independent association between rheumatoid arthritis (RA) and periodontitis (PD). Both are chronic inflammatory diseases characterised by an exacerbated immune-inflammatory reaction that leads to the destruction of bone and other connective tissues that share numerous similarities. Hence, the biological mechanisms underpinning such a relationship have been a focus of investigation for many years.[1] However, the pathophysiological processes that may explain this relationship remain unknown and multiple theories have been proposed.

Given the increasing number of clinical studies suggesting the beneficial effects of periodontal therapy on RA outcomes, there is a need for more studies aimed at understanding the mechanisms that connect these two conditions and to help explain why periodontal therapy may be beneficial. This review aims to lay out the most important theories proposed to explain the biological link between RA and PD.

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Risk factors

RA and PD have multiple risk factors in common (Table 1) and it has therefore been hypothesised that the epidemiological relationship between the two conditions could possibly be explained by the 'common risk' hypothesis.

Genetic risk factors

RA and periodontitis have certain common genetic factors impacting upon the host's immune response that translate into, for example, higher levels of pro-inflammatory cytokine production. The human leucocyte antigen, allele DR4 (HLA-DR4) epitope is located on the surface of leucocytes and has been found to be associated with both RA and periodontitis.[2]

In common with several other immune-mediated diseases, numerous studies report an association between HLA genes and periodontitis. The most recent systematic review reported a protective association with HLA-A2 and B5, and an increased susceptibility for periodontal disease with HLA-A9 and B15 genotypes.[3] While classical twin studies have shown that periodontitis exhibits a genetic component,[4] genome wide association studies (GWAS) have been unable to identify significant associations with chronic periodontitis.[5] A recent systematic review of 43 studies concluded that there was no evidence for an interaction between any genetic variants (including IL1) and the sub-gingival microflora.[6]

In RA, robust data are available from genetic association studies, suggesting HLA SE (shared epitope) alleles as the main candidate gene in anti-citrullinated protein antibody (ACPA) positive patients.[7] In addition to these alleles, GWAS have revealed another 32 loci associated with RA.[8] There is also a clear dissimilarity between genetic risk factors for ACPA-positive and ACPA-negative RA, thus more research is required to understand the genetic differences between these two groups.[9]

There are, however, currently few studies investigating a genetic link between RA and periodontitis[10,11,12] and no GWAS studies. Therefore, the importance of genetic polymorphisms in the link between these two diseases remains unknown.

Smoking

Smoking is a shared modifiable risk factor for both periodontitis and RA,[13] with recent data demonstrating a threefold increased risk of RA in ACPA-positive men who smoke (1.8-fold increase for women smokers), although no association was found in ACPA-negative patients.[14] This association with smoking is not, however, evident in patients with early rheumatoid arthritis. This group of patients exhibit a high prevalence of periodontitis but have a low prevalence of smoking (16%) and a young age (mean age 42.2 years old).[15] In contrast, some scientists have reported a significantly higher risk of periodontitis in non-smokers with RA,[16] whereas others have found an association between RA and periodontitis irrespective of smoking status.[17,18]

Therefore, although periodontitis and RA share genetic (HLA-DR4) and lifestyle (for example, smoking) risk factors, as well as similar inflammatory pathways, these do not appear to be sufficient to explain the connection between the two diseases.[19,20]

Table 1 Shared risk factors between periodontitis and rheumatoid arthritis (RA). Abbreviations as follows, PMNL: polymorphonuclear leucocyte; CT: connective tissue

Periodontitis	RA
Smoking	□
Genetics	□
Host-mediated CT destruction	□
A role for plasma cells in active disease	□
PMNL infiltration = substantial	□
Oxidative stress = major feature	□
Female sex hormones play a role	□
Symptoms respond to anti-inflammatory drugs	□

Fig. 1 Patient with rheumatoid arthritis (RA) holding a toothbrush, showing the typical hand deformities of severe RA sufferers

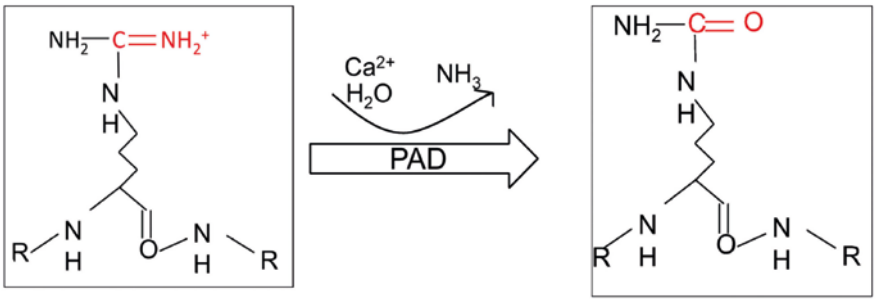

Fig. 2 Protein citrullination by peptidyl-arginine deiminase (PAD). In the presence of calcium, PAD catalyses the conversion of arginine in to citrulline, changing the ketamine group (= NH) for a ketone group (= O)

Poor oral hygiene

Poor oral hygiene has also been proposed as a link between rheumatoid arthritis and periodontitis. The hypothesis that RA patients are unable to maintain good oral hygiene compared to healthy controls due to impaired manual dexterity is attractive as an explanation for the association between the two. Indeed, it is logical that the hand and joint deformities evident in the most severe forms of RA, alongside the associated functional limitations, predispose to poor levels of plaque removal, and therefore an increased risk of periodontitis (Fig. 1). However, studies have consistently failed to show a difference in plaque control that could explain the association with periodontitis.[21,22,23]

Gender

One of the biggest disparities we find between the incidence of RA and periodontitis is the gender frequencies. While 75% of RA patients are female, men are believed to have worse periodontal health.[24] This gender difference has not been fully investigated, although it may well be explained by a stronger effect of smoking and obesity in the development of periodontitis. However, it is known that in both diseases female hormones play a role, worsening periodontal status in female puberty, pregnancy and at the postmenopausal stage.[25]

Protein citrullination

Protein citrullination or 'deimination' has been widely studied due to its role in autoimmune diseases, especially in RA. This post-translational modification of proteins occurs when a family of enzymes called peptidyl-arginine deiminase (PAD) transforms the amino acid arginine into citrulline. In the presence of calcium, PAD replaces the ketamine group (= NH) with a

ketone group (= O), converting the positively charged arginine into a neutrally charged citrulline amino acid.[26] This renders the parent protein hydrophobic, resulting in a change in protein folding and thus function (Fig. 2).

While citrullination is a physiological phenomenon in healthy individuals, in RA citrullinated proteins are recognised as an antigen and activate the immune system.[27] Therefore, why the immune system produces antibodies against citrullinated proteins in RA patients remains unknown and this unique production of auto-antibodies in RA suggests the necessity for an external factor to trigger this autoimmune reaction. It has also been shown that PAD (in particular PAD-2 and PAD-4) are expressed in inflamed periodontal tissues and this may be one important source of protein citrullination, arising many years before the RA develops.[28] This event could break the immune tolerance to citrullinated proteins and predispose to RA following a second insult in the joints themselves.

Porphyromonas gingivalis citrullination

Porphyromonas gingivalis (*P. gingivalis*) is gram-negative anaerobic bacteria strongly associated with periodontitis which is considered key to disruption of the host-microbial homeostasis that characterises the disease.[29,30,31] Interestingly, *P. gingivalis* uniquely expresses peptidyl arginine deiminase (PPAD) which is capable of citrullinating both host and bacterial peptides[32] for its survival within the periodontal pocket.[33] It has been observed in some *in vitro* studies that PPAD can only citrullinate C-terminal arginine and not internal arginine, in contrast to its homologues human PAD-2 and PAD-4, creating citrullinated peptides that would not normally occur in the absence of *P. gingivalis*. By presenting these neoepitopes to the immune system, *P. gingivalis* could break immune tolerance to citrullinated proteins and lead to the subsequent generation of ACPAs characteristic of RA patients (Fig. 3).

The potential role of *P. gingivalis* in the aetiology of RA has been investigated over the last decade.[34] Martinez-Martinez *et al.*[35] found DNA from *P. gingivalis* in the synovial fluid of patients with RA and established that periodontal pathogens could be a trigger for the autoimmune response in RA. However, PPAD itself to date has not been found within synovial fluid. Hitchon *et al.*[36] reported an association between immune responses to *P. gingivalis*

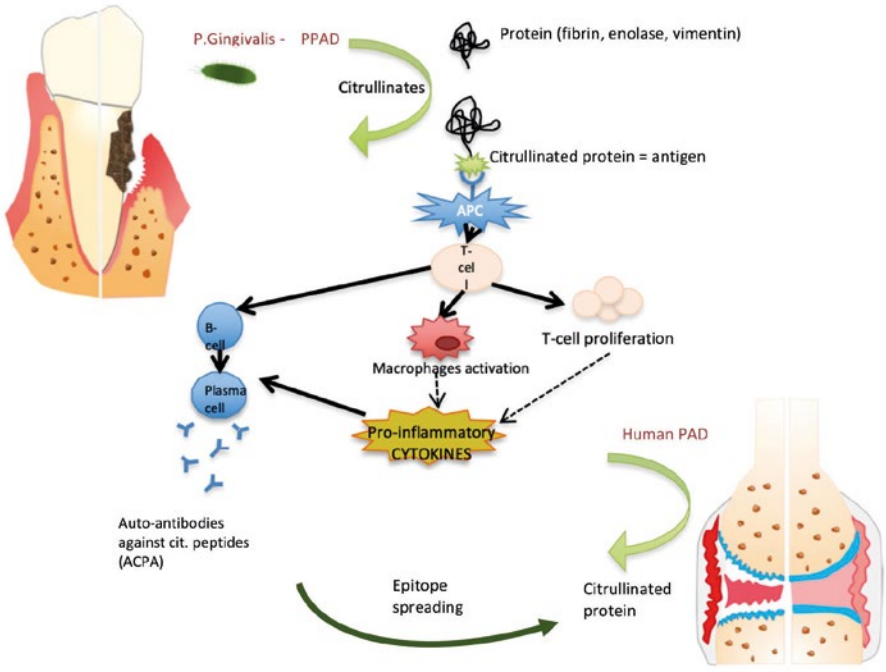

Fig. 3 Model of the biological link between rheumatoid arthritis and periodontitis. *P. gingivalis* breaking immune tolerance to citrullinated proteins in rheumatoid arthritis (RA) by bacterial citrullination of proteins through PPAD, initiating an immune response leading to loss of tolerance to citrullinated proteins in the joints. Abbreviations as follows, Porphyromonas gingivalis: *P. gingivalis*; PPAD: peptidyl arginine deiminase from *P. gingivalis*; APC: antigen presenting cell; TNF: tumour necrosis factor; IL: interleukin; GMCSF: granulocyte-macrophage colony-stimulating factor

and the presence of ACPA in a population with a high background prevalence of the RA predisposing HLA alleles. A study by Mikuls *et al.*, showed that high levels of anti-P. *gingivalis* antibodies in RA subjects correlated with levels of ACPAs, suggesting that this organism plays a role as a risk factor in RA.[37]

Interestingly, it has been observed that the periodontium of periodontitis patients with no signs of RA express citrullinated proteins[38] and that serum from these patients contain higher levels of antibodies to citrullinated and non-citrullinated human peptides compared to healthy controls.[39] De Pablo *et al.* suggested that the greater citrullination arising in periodontitis leads to a loss of tolerance to citrullinated and un-citrullinated peptides, which may evolve in a cross-reaction against the citrullinated proteins in the joint that may in turn lead to RA.[39]

Based on these findings, researchers propose that bacterial and human PADs could be a therapeutic target in RA.[40] Moreover, it has been hypothesised that periodontal therapy could decrease the load of *P. gingivalis* and PPAD in the periodontal pocket and that, by reducing gingival inflammation, the expression of human PADs could be reduced, weakening the autoimmune response in RA.

However, it remains unclear whether there is a causal role for antibodies to citrullinated proteins in the evolution of RA, and there is currently no strong evidence that periodontal therapy reduces citrullination systemically .

Other periodontal bacterial candidates to play a role in RA

Recently, other periodontal pathogens have been identified as possible triggers for RA. In a study conducted by Konig *et al.*, the authors found that *Aggregatibacter actinomycetemcomitans* was the only bacteria able to induce hypercitrullination within human neutrophils, when investigating a number of periodontal pathogens and oral commensals. Through secretion of leukotoxin A (LtxA), *A. actinomycetemcomitans* induces calcium influx and hyper activation of PAD in the neutrophil, leading to neutrophil hypercitrullination.[41] Interestingly, the effect of human lymphocyte antigen-DRB1 shared epitope alleles on auto-antibody positivity was limited to RA patients who were exposed to *A. actinomycetemcomitans*.

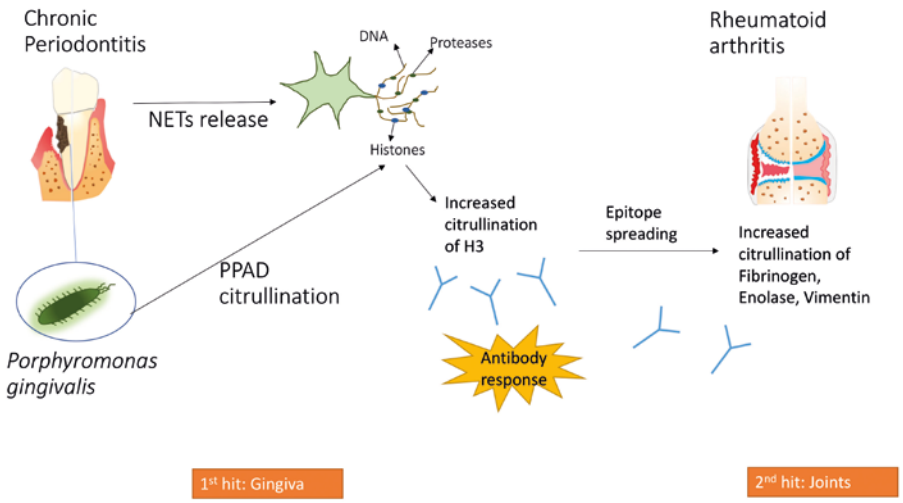

Fig. 4 Hypothesis of the role of NETs in the connection between RA and periodontitis. Abbreviations as follows NETs: neutrophil extracellular traps; PPAD: peptidyl-arginine deiminase from *Porphyromonas gingivalis*; H3: histone 3

Last year, a study investigating the oral microbiome in RA identified *Cryptobacterium curtum* (previously misclassified as *Eubacterium saburreum)* as a predominant member of the RA-influenced periodontal microbiome with a 100-fold greater abundance in RA and with 39-fold greater odds of detection when compared to non-RA controls.[42] Interestingly, this gram-positive anaerobic rod/periodontal pathogen[27] degrades arginine through the arginine deiminase pathway and produces substantial amounts of citrulline, ornithine and ammonia.[43] Others have previously found *C. curtum* to be enriched in the oral and gut microbiomes of early RA cases.[15,44] Thus, the authors suggest that *C. curtum* is a candidate for further studies.

Citrullinated human proteins targeted in RA

Anti-citrullinated protein antibody (ACPA) testing kits are regularly used by rheumatologists to measure antibodies against citrullinated proteins and to diagnose patients as ACPA + and ACPA - which can be used to anticipate disease severity and treatment responses.[45] This test uses a synthetic citrullinated cyclic peptide as an antigen to capture antibodies against any citrullinated proteins present in patient sera. Moreover, investigating the proteins that are citrullinated in RA can help us understand the development of the autoimmune response and development of the disease. To date, numerous studies have reported that proteins including fibrinogen, vimentin, enolase and tenascin are targeted in RA and antibodies against these citrullinated proteins are associated with the severity of the disease and also elevated in periodontitis patients.[45,46,47,48,49,50]

The current data demonstrate that both RA and periodontitis induce citrullination, which may be the link between these two diseases. Recently, it has been found that the production of citrullinated proteins in the periodontal tissues is associated with gingival inflammation,[28] occurring in 80% of periodontitis-affected stroma.[38] This is supported by the discovery of higher serum antibodies against citrullinated proteins in periodontitis patients compared to controls.[39] For this reason, investigators believe that the gingiva of periodontitis patients might be an extra-articular source of citrullinated proteins, which can lead to ACPA production contributing to RA progression.

Carbamylation and malondialdehyde-acetaldehyde adducts

As detailed above, post-translational modification of proteins can lead to the production of autoantibodies and loss of immune tolerance. Apart from citrullination, several other post-translational modifications of proteins have been associated with RA. Of these, malondialdehyde-acetaldehyde adducts and carbamylated proteins are receiving particular attention.[51] A recent study identified immunohistochemical evidence of the presence of malondialdehyde-acetaldehyde adducts, citrullinated and carbamylated proteins in inflamed human gingival tissue biopsies but not in biopsies of healthy, non-inflamed gingivae.[52] The identification of such modified proteins in inflamed gingiva suggests that inflammation of the periodontal tissues may influence the development of RA.

Porphyromonas gingivalis antigens targeted in RA

Proteins from *P. gingivalis* may be recognised as an antigen by the host and initiate an immune response that could break tolerance to human antigens such as citrullinated proteins. Based on this hypothesis, researchers have investigated antibodies against some *P. gingivalis* antigens and their role in RA.

Antibodies against citrullinated PPAD (CPP3 and CPP5) are increased in ACPA+ RA patients as well as in periodontitis patients (CPP5).[53] In RA, and in patients in the pre-clinical period of the disease (pre-RA patients), anti-CPP3 antibodies are elevated,[54] although other researchers have been unable to find an association between anti-PPAD antibodies and early RA.[55] Another limitation of this hypothesis is the lack of studies investigating the presence of PPAD in inflamed periodontal tissues from RA patients.

While only a few studies have investigated these antibodies and more research is needed, considering these results, the hypothesis that *P. gingivalis* antigens may break immune tolerance in RA, requires further investigation and, if proven, these epitopes could be targeted in novel therapies for RA patients.[53]

The role of the oral microbiome in RA

The human microbiome is defined as 'the genes carried by the particular community of microorganisms that live in and on the human body'. Using DNA-based technologies to investigate bacteria has opened a new chapter in our understanding of the microbes that live with us,[56] as traditional cultivation techniques limited understanding to the minority of organisms that could be grown outside the body.

Currently, three microbiome studies have investigated the oral microbiome in RA patients. [15,57] Zhang *et al.* reported the sub-gingival, oral and gut microbiome to be altered in RA, and certain species such as *Lactobacillus salivarius* and *Cryptobacterium curtum* were enriched, therefore offering

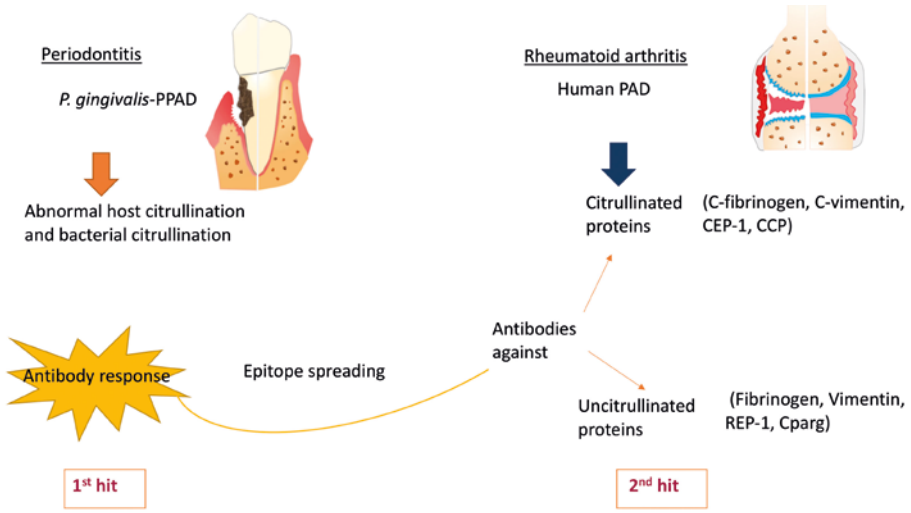

Fig. 5 Two hit model proposed to explain the link between periodontitis and RA. Abbreviations as follows, PAD: peptidylarginine deiminase; PPAD: peptidylarginine deiminase from *Phorphyromonas gingivalis*; Cit: citrullinated; CEP: enolase 1; REP: non-citrullinated enolase 1; Cparg: negative control for anti-CCP; CCP: anti-cyclic citrullinated protein

potential diagnostic tools.[57] In a study of early RA patients, Scher and colleagues noted that some oral bacterial taxa were significantly different in abundance, however overall measures of diversity were no different to systemically healthy controls. In a more recent study, investigating the periodontally healthy oral microbiome in RA, it was found that compared to controls, RA patients had a distinct microbiome with a significantly higher percentage of anaerobes, including *Cryptobacterium curtum*.[41] However, the effect of periodontal therapy on the oral microbiome remains to be fully investigated using next generation sequencing techniques, and the effect in RA patients remains unknown.

The cytokine imbalance in RA and periodontitis

In RA, there is an imbalance between the pro-/anti-inflammatory host systems,[58] similar to that reported in periodontitis patients.[59] Understanding the cytokine networks involved in the pathobiology of RA has led to a revolution in the therapeutic arsenal available for this disease in the last decade[60] and may also represent a key biological link between RA and periodontitis.

To date, several studies have evaluated the effect of TNF inhibitors in periodontitis and RA.[61,62,63,64,65] Although the authors concluded that anti-TNF therapy could benefit periodontal status, they suggest that it is difficult to predict the collateral harm that may result from targeting cytokines therapeutically.[66]

Interestingly, periodontal therapy has been shown to reduce serum TNF levels in some studies,[67,68] while other studies failed to find this reduction.[69] For this reason, it is important to investigate periodontal therapy as a potentially safe and non-pharmacological treatment that could reduce inflammation in systemic inflammatory conditions such as RA.[70,71,72]

Neutrophils and neutrophil extracellular traps

Neutrophils are the first leucocyte to arrive at the site of infection and their principal functions include immunological surveillance, recognising and phagocytosing microorganisms, and activation of and collaboration with humoral immunity. When activated by multiple inflammatory signals, neutrophils may release their enzymatic content to the exterior of the cell, together with their DNA, forming a web-like structure called a neutrophil extracellular trap (NET), whose purpose is to immobilise microbes and prevent their spread. Although effective, the release of these contents, along with reactive oxygen species (ROS), provokes collateral tissue damage to the surrounding tissues, exacerbates the inflammatory response and exposes possible autoantigens.[73,74]

Elevated NET formation occurs in both RA and periodontitis patients,[75,76] and there is a clear correlation between levels of DNA in the joints, serum ACPA levels and neutrophil counts, as well as a clear mapping of citrullinated proteins to neutrophils within cell pellets prepared from inflamed joints. NET release was also reported to be correlated with high ACPA and rheumatoid factor (RF) levels.[77]

Taken together, these results suggest that, in periodontitis, a chronic exposure of gingival tissues to PAD-4 and citrullination may result in the breakdown of immune tolerance to citrullinated peptides in susceptible individuals which could lead or contribute to RA pathogenesis. NET generation may therefore not only contribute to periodontitis and RA pathogenesis, but it also provides a potential causal link between these two conditions (Fig. 4).[78]

The 'two-hit' model of RA pathogenesis

Golub *et al.* were the first to describe a theory in which the association between periodontitis and systemic diseases could be explained by a 'two hit' model.[79] In this theory, a first 'hit' arises within the periodontum, initiated by an infection that activates a destructive inflammatory cascade in the periodontal tissues. In susceptible patients, a second systemic 'hit' then occurs, characterised by increased serum levels of pro-inflammatory cytokines that amplifies the inflammatory cascade, with production of local and systemic pro-inflammatory mediators (cytokines and prostaglandins).

A similar 'two hit model' was described six years later to explain the breakdown of immune tolerance to citrullinated proteins in RA patients triggered by smoking.[80] Similar to smoking, periodontitis could represent a first hit of citrullinated peptide production. In a first extra-articular hit, chronic periodontitis could break immune tolerance to citrullinated proteins, due to abnormal levels of protein citrullination within periodontal tissues (the periodontal citrullinome). Through epitope spreading (a process in which the immune response does not remain fixed towards a specific epitope, but extends to include other epitopes on the same protein or other proteins in the same tissue), this local autoimmune response to citrullinated proteins could lead to the production of ACPAs systemically. These ACPAs react against citrullinated proteins in the joint since, in joint diseases there is an increase of protein citrullination in the

synovium. This model describes a primary 'hit' of ACPA production due to chronic periodontitis followed by a secondary 'hit' in the joint, that could induce RA (Fig. 5).[19,81]

Summary and conclusions

Although promising, the theories described in this paper to link RA and periodontitis remain to be proven and studies supporting them have limitations. None of the theories described above explain fully why this inflammatory response, initiated or exacerbated by periodontitis, would be specific to the joint structures in RA, and it remains unproven whether this link is coincidental or due to common risk factors. A causal relationship between RA and periodontitis cannot be established with the current evidence, and if proven, the relationship may only exist in a subset of RA patients.

Therefore, future research investigating the oral microbiome, inflammatory and autoimmune response of pre-RA patients, RA patients and periodontitis patients, is required to clarify gaps in knowledge. Depending upon the outcomes of such studies, therapies targeting the microbiome/specific microorganisms or components of the immune response could help to prevent both diseases.

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