

Potential Mechanisms Linking Periodontitis to Rheumatoid Arthritis

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Abstract

Periodontitis (PD), a chronic inflammatory disease which results in irreversible attachment loss, bone destruction and tooth loss, is a major oral health problem. Rheumatoid arthritis (RA), with a global prevalence of 1%, is an autoimmune disease characterized as a chronic inflammatory disorder leading to synovial inflammation and destruction of cartilage and bone. Studies have reported an association between PD and RA whereby PD is reportedly more severe in patients with established RA. Justification for the plausible link between both conditions is based on shared characteristics and pathogenic similarities with regard to risk factors, immuno-genetics and tissue destruction pathways. The search for the possible mechanism linking PD to RA continues as it can play an important role in enabling early intervention in the form of prevention and treatment of infection. This will ultimately improve patients' oral health related quality of life and reduce societal burden related to increased patient discomfort and treatment costs. The current review provides an update on the cellular and molecular events that have thus far explained the link.

Keywords: *rheumatoid arthritis, periodontitis, Porphyromonas gingivalis, Peptidyl Arginine Deiminase, carbamylation, citrullination*

Introduction

Periodontitis (PD) is a chronic inflammatory disease causing irreversible attachment loss, bone destruction and tooth loss. There are two major forms of periodontitis: chronic periodontitis and aggressive periodontitis. Chronic periodontitis is slowly progressive whereas aggressive periodontitis is rapidly progressive with extensive periodontal destruction in younger patients. The primary aetiology of PD is the dental biofilm (Löe *et al.*, 1965) while the host inflammatory response causes the resulting tissue damage (Bartold *et al.*, 2013) (Figure 1).

Rheumatoid arthritis (RA) is an autoimmune disease characterized as a chronic inflammatory disorder leading to synovial inflammation and destruction of the cartilage and bone. Prior to the clinical manifestations of RA, a preclinical immunological phase shown by the identifica-

tion of serum autoantibodies is seen in some patients years before the development of RA (Demoruelle *et al.*, 2014) (Figure 2).

It is now well evident that periodontitis is more common and severe in patients with established RA (Bartold *et al.*, 2005; Chen *et al.*, 2013; De Pablo *et al.*, 2009; Dissick *et al.*, 2010; Scher *et al.*, 2012). While patients with RA have more advanced periodontitis than the general population (De Pablo *et al.*, 2009; Dissick *et al.*, 2010), individuals with moderate to severe periodontitis are at a higher risk of suffering from RA and vice versa (Kaur *et al.*, 2013). The current review describes the cellular and molecular events that contribute to the potential mechanisms involved in linking PD to RA.

Common Immuno-genetics Shared between RA and PD

Both RA and PD are characterized by the overproduction of pro-inflammatory cytokines such as TNF- α , IL-6 and IL-1 (Bıyıkoglu *et al.*, 2006; Mirrieles *et al.*, 2010). Both diseases also share common risk factors such as smoking, age and poor oral hygiene due to loss of dexterity (Agnihotri *et al.*, 2014; Detert *et al.*, 2010).

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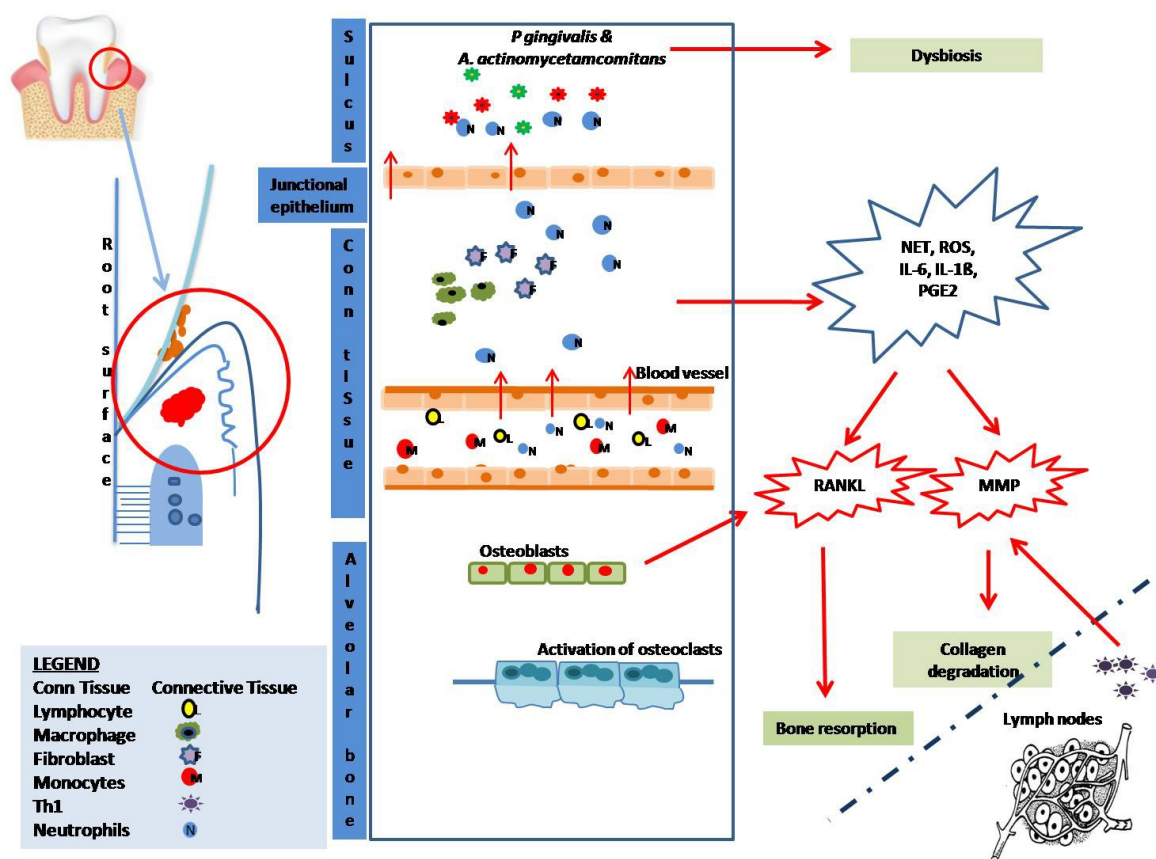


Figure 1. An overview of the cellular and molecular aspects of the chronic inflammatory response in periodontitis based on the current understanding of disease mechanisms.

The immuno-pathogenesis of periodontitis involves both innate and adaptive immune systems. *P. gingivalis* acts as the antagonist in disrupting the homeostatic balance between the subgingival commensal microflora and the host defense system. Lipopolysaccharide (LPS), the cell surface protein of *P. gingivalis* and *A. actinomycetemcomitans*, is recognized by toll-like receptors (TLRs) found on resident cells including epithelial cells and mast cells. The activated resident cells release IL-8, creating a chemotactic gradient that drives the trans-endothelial migration of neutrophils towards the infection site (gingival sulcus) to phagocytose the microbes. Neutrophils can also kill microbes by releasing neutrophil extracellular traps (NETs), during which lysosomal enzymes i.e. myeloperoxidase and matrix metalloproteinase (MMP) are aborted to destroy the microbial colony. However, these enzymes can revert back into the tissues and cause collagen degradation. The increased production of the reactive oxygen species (ROS) following NETosis can stimulate receptor activator of the nuclear factor kappa-B ligand (RANKL) expression on osteoblast and trigger osteoclast activation. The function of fibroblasts is also altered during inflammation. When stimulated by inflammatory cytokines, gingival fibroblasts secrete MMPs which causes more tissue destruction. The persisting dental biofilm enhances the generation of pro-inflammatory cytokines such as IL-6 and TNF- α , resulting in the migration of lymphocytes to thwart the microbial insult. Activated T and B lymphocytes express RANKL and induce osteoclast differentiation, leading to bone resorption. As inflammation intensifies, the infiltration of inflammatory cells increases and the net effect of all these pathways is progressive tissue destruction.

Based on such similarities in host response, it is likely that both diseases share the same genetic background. Hence, susceptibility to RA and PD may share similarities regarding genetic risk factors.

Genetic studies related to RA

Genetic and environmental factors play a modifying role on the host response in the pathogenesis of RA. The first genetic risk factor identified for RA is the human leukocyte antigen (HLA) locus, whereby it was noted that 78% of Caucasian RA patients were HLA-DRw4 positive in comparison with 28% of healthy controls

(Stastny, 1978). In 1987, the 'shared epitope' hypothesis emerged where it was found that a specific region (positions 70-74) of the HLA-DRB1 gene shared a conserved amino acid sequence known as the shared epitope (SE) alleles (Gregersen *et al.*, 1987). HLA-DRB1 is part of the HLA gene family that plays a central role in immune system. Differences amongst ethnic groups have been reported whereby HLA-DRB1 is only found in 25% of African-American patients, HLA-DRB1*0405 is the most frequent allele in Asian RA patients and HLA-DRB1*1402 is associated with RA in Native American patients (Bodis *et al.*, 2018; Hughes *et al.*, 2008).

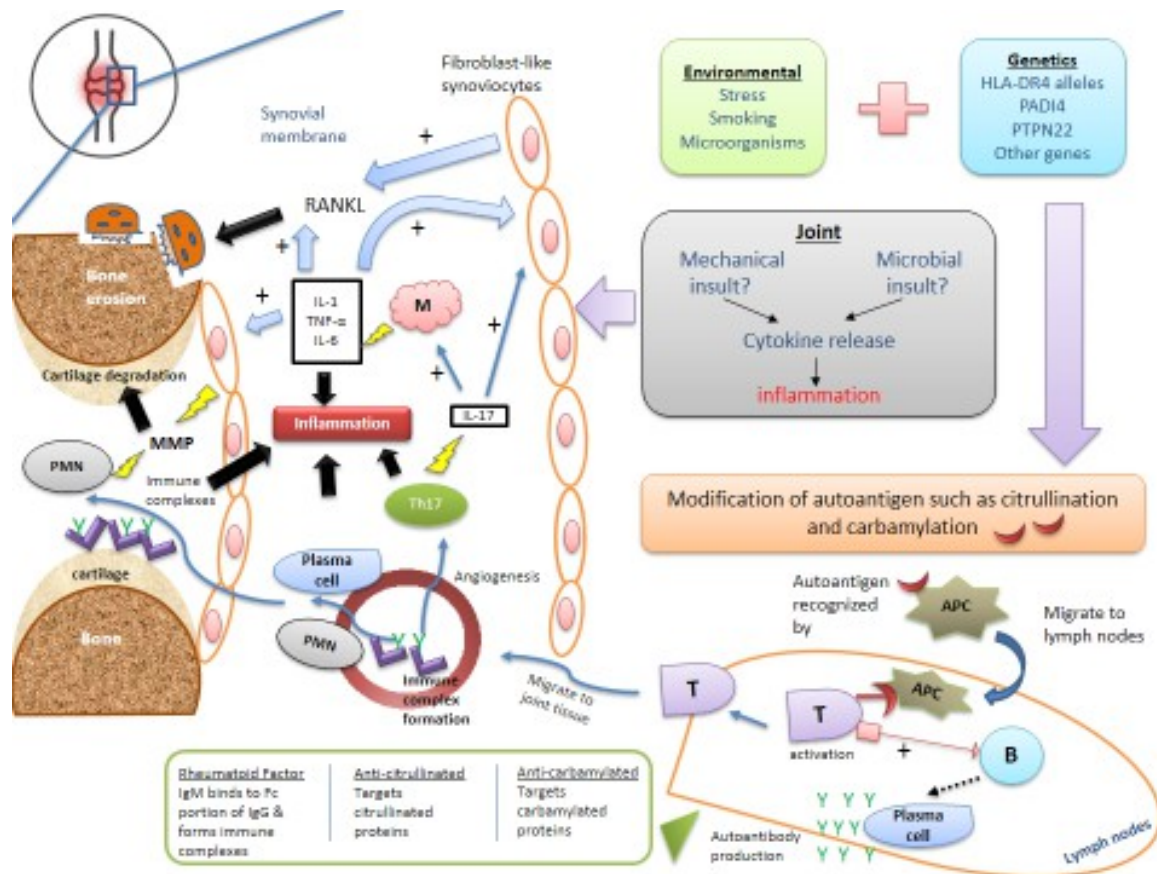


Figure 2. A schematic representation of pathogenic pathways in rheumatoid arthritis.

RA is an autoimmune disorder of unknown cause. Current evidence suggests that a combination of genetic and environmental risk factors are involved in the pathogenesis of RA. Certain environmental triggers such as stress, smoking and infectious agents can precipitate post-translational modification of proteins as a physiological process but the modified proteins may break the immune tolerance in genetically susceptible individuals, leading to autoantibody production. The circulating autoantibodies form immune complexes with autoantigens, and in the event of trauma or infection, the immune complexes gain access to the synovial space through dilated vessels. As a result, the classical complement pathway is activated and leukocyte recruitment is stimulated. The pro-inflammatory cytokines generated by immune cells alters the metabolism of fibroblast-like synoviocytes (FLS). Instead of synthesizing extracellular matrix components, FLS secrete matrix metalloproteinases and cytokines that degrade the cartilage tissue and induce osteoclastogenesis. The cytokines released further enhance the recruitment of leukocytes and thus, creating a positive feedback loop. The resultant event is the persistence of chronic inflammatory response within the joint which eventually leads to irreversible soft and hard tissue destruction.

Peptidylarginine deiminase type 4 (PADI4), a gene outside the HLA region was identified as the second genetic risk factor for RA which is significant in Asians but not in people of European descent (Suzuki *et al.*, 2003). Furthermore, an enhanced peptide affinity for MHC (major histocompatibility complex) class II molecules containing the SE was demonstrated by the conversion of arginine to citrulline at the peptide side-chain position in HLA-DRB1*0401 transgenic mice. This led to the hypothesis that post-translational modification of arginine might be responsible for T cell activation to initiate an autoimmune response in RA patients (Hill *et al.*, 2003).

While HLA SE alleles remain the main candidate genes in RA susceptibility, they only account for 11% of the total genetic variance of RA which was much lower

than previously thought (van der Woude *et al.*, 2009). With the recent application of genome-wide association studies (GWAS), in addition to HLA SE alleles, more than 32 non-HLA risk loci have been identified to be involved in the pathogenesis of RA (Chatzikyriakidou *et al.*, 2013) and the number is rising owing to more GWAS being conducted in different ethnic populations around the world. Besides, a pilot study by Padyukov *et al.* (2011) showed significant dissimilarity in genetic risk factors between anti-citrullinated peptide antibodies (ACPA) positive and ACPA negative subgroups. Most studies have focused on ACPA positive RA subjects. Newer studies need to incorporate more investigations of ACPA negative patients to help decipher the pathogenesis of ACPA negative RA.

Genetic studies related to PD

Similar to RA, genetic predisposition is one of the recognized risk factors for PD, with aggressive periodontitis displaying a strong familial aggregation (Novak *et al.*, 1995). Twin studies have previously shown that periodontitis has a genetic component (Ciancio *et al.*, 1969; Corey *et al.*, 1993; Michalowicz *et al.*, 1991). Michalowicz *et al.* (2000) confirmed that adult periodontitis had more than 50% heritability (Michalowicz *et al.*, 2000). Through candidate gene studies or GWAS, a total of 38 genes have been identified including the discovery of some novel candidate genes which were previously thought unrelated to periodontitis (Vaithilingam *et al.*, 2014). Despite the high number of risk genes reported, only a fraction of susceptibility genes such as *ANRIL*, *GLT6D1* and *COX-2*, which are mainly associated with aggressive periodontitis, have been validated as conclusive risk loci of periodontitis (Vaithilingam *et al.*, 2014). Although no specific gene or locus has been found to be significantly associated with chronic periodontitis in large-scale GWAS (Divaris *et al.*, 2013; Teumer *et al.*, 2013) 3 genes, namely *VDR*, *Fc-γRIIA* and *IL-10*, display a strong level of association (Chapple *et al.*, 2017). However, varied single nucleotide polymorphisms (SNPs) were observed within these genes with significant population differences. For instance, different SNPs in the *VDR* gene were reported in Chinese (rs731236) and Japanese (rs2853564) populations (Wang *et al.*, 2009). Currently, a moderate role has been attributed to the genetic component cause to periodontal diseases (Chapple *et al.*, 2017). Thus, further studies with stringent case definition and diverse populations are necessary to clarify the SNPs and their strength of association with different populations.

Genetic studies related to RA-PD

Reports on the common genetic factors shared by both RA and PD are scanty (Table 1). While investigating the common HLA associations in patients with juvenile idiopathic arthritis and either chronic periodontitis or with aggressive periodontitis, a positive association was observed between HLA-DRB3* and juvenile idiopathic arthritis and chronic periodontitis (Reichert *et al.*, 2007). The small sample size of aggressive periodontitis subjects might have resulted in the lack of association with HLA-DRB3* (Reichert *et al.*, 2007). While addressing the association between PD and joint destruction in RA, individuals with SE were found 3.9 times more likely to have both joint and periodontal destruction than SE negative individuals (Marotte *et al.*, 2006). Since SE negative individuals also presented with RA, SE was suggested as a severity marker rather than an etiological factor. One of the weaknesses of this study was the potential under-diagnosis of PD among the participants. This was because the inclusion criteria for PD diagnosis was based on radiographic assessment of only four molar teeth.

Studies on cytokine gene polymorphism revealed shared cytokine profile of both RA and PD. For example, RA and aggressive periodontitis patients were found to have the same genetic background of *IL-1* among Danish white population (Havemose-Poulsen *et al.*, 2007). A higher distribution of *IL-1B*+3954 single nucleotide polymorphism (SNP) was found associated with RA and PD in Japanese population compared with PD alone and healthy subjects (Kobayashi *et al.*, 2010). Similarly, a stronger link between *IL-1B*-511 SNP and both diseases (RA and PD) was observed among a Mexican population

Table 1. Genetic studies related to RA-PD

Authors	Type of study	Ethnicity	Number of subjects		Common genetic components of RA and PD
			Case	Control	
Marotte <i>et al.</i> , 2006	Case-control (SE+ve vs SE-ve)	French	RA-PD: 73	RA: 64	Disease severity increases in SE+ve
Reichert <i>et al.</i> , 2007	Case-control (Females with JIA, CP vs controls)	Germans	JIA: 59 CP: 63	Healthy: 62	HLA-DRB3*
Havemose-Poulsen <i>et al.</i> , 2007	Case-control	Danish Caucasian			<i>IL-1</i> genotypes
Kobayashi <i>et al.</i> , 2009	Case-control	Japanese	RA-PD:137 PD: 117	Healthy: 108	<i>IL-1B</i> +3954
Domínguez-Pérez <i>et al.</i> , 2017	Case-control	Mexican	RA-PD: 40 RA: 80 PD: 80	Healthy: 80	<i>IL-1B</i> -511
Kobayashi <i>et al.</i> , 2018	Case-control	Japanese	RA-PD: 185 CP: 251	Healthy: 130	KCNQ1 rs2237892

SE: shared epitope; JIA: juvenile idiopathic arthritis; RA: Rheumatoid arthritis; PD: Periodontitis; CP: chronic periodontitis; +ve: positive; -ve: negative

(Domínguez-Pérez *et al.*, 2017). In a recent study on the Japanese population, KCNQ1 rs2237892 was found to be significantly associated with comorbidity of RA and CP after adjustments were done for age, sex, and smoking status (Kobayashi *et al.*, 2018).

Thus far, a definite answer regarding the shared genetic polymorphisms between both RA and PD has yet to be established. It is possible that multiple gene variants are involved, with each gene variant contributing a small percentage of cases. Therefore, a hypothesis-free, genome-wide search will be more effective in assessing the common risk genes associated with both RA and PD.

***Porphyromonas gingivalis* as a Key Player in Linking PD to RA**

The presence of periodontal pathogens in the subgingival biofilm has been known to be a vital contributing factor in the initiation and progression of periodontitis. The most virulent pathogens related to chronic periodontitis are *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* (Socransky *et al.*, 1998). *P. gingivalis* has been the subject of particular interest among researchers in the last decade owing to its unique properties that allow it to escape from the host defense mechanisms and manipulate the complement pathway to its own benefit.

In 2004, Rosenstein and co-workers postulated that PD preceded the onset of RA whereby *P. gingivalis* played a central role in driving the production of autoantibodies against self-antigen expressed in the inflamed joints (Rosenstein *et al.*, 2004). The assumption that oral bacteria could trigger the development of RA arose following the discovery of Moen *et al.*, whereby the trapping of *P. gingivalis* DNA within the synovial fluid of inflamed joints was observed in RA subjects (Moen *et al.*, 2006). Martinez-Martinez *et al.* (2009) reported that 57.8% of *P. gingivalis* DNA was detected in the synovial fluid sample of RA patients and the migration of the bacterial DNA from the oral cavity to the joint could be in free DNA form. Although the sample size of both studies was small, they gave new insights into the plausible aetiological link between periodonto-pathogens and RA. A more recent study with a slightly larger sample size also documented the presence of *P. gingivalis* DNA in the synovial tissue and fluid of RA patients (Totaro *et al.*, 2013). The study reported that the amounts of *P. gingivalis* DNA detected was significantly higher in the synovial tissue than in the synovial fluid.

Apart from the detection of bacterial genetic material, some studies tested the immune response to *P. gingivalis* in RA samples. Chukkapalli *et al.* (2016) induced PD in genetically collagen-induced arthritis (CIA) prone B10-RIII mice with 3 different bacterial species (*P. gingivalis*, *T. denticola* and *T. forsythia*) for 24 weeks. They noted a significant elevated antibody level to periodontal

pathogens in infected mice than uninfected mice but only *P. gingivalis* DNA was detected in the inflamed joints of infected mice. This supports the hypothesis that *P. gingivalis* may be able to amplify autoimmune arthritis through systemic dissemination. The same hypothesis was shared by Kharlamova *et al.* (2016) (Kharlamova *et al.*, 2016), in which a large epidemiologic assay of an immune response to *P. gingivalis* in patients with early onset RA was performed. The data showed 23.1% of RA patients had increased anti-*P. gingivalis* antibody levels compared to 9.6% of non-RA controls. This was the first observational study conducted using a large sample size and a more specific antibody as a serological marker to speak volumes about their results. The *P. gingivalis* link between PD and RA was further endorsed by a current systematic review that infers RA patients tend to show a significantly higher antibody response to *P. gingivalis* than systematically healthy individuals, irrespective of the presence of PD (Bender *et al.*, 2017).

However, other studies conducted by Milkus and colleagues failed to detect any significant differences in anti-*P. gingivalis* antibody levels between RA patients and controls (Mikuls *et al.*, 2018) and between RA and non-RA patients with no or moderate periodontitis (de Smit *et al.*, 2012). There was however significantly higher anti-*P. gingivalis* titers in RA patients with severe periodontitis than the matched non-RA patients although subgingival occurrence of *P. gingivalis* was not different (Scher *et al.*, 2012). These conflicting findings may be explained by differences between RA cohorts and also differences between *P. gingivalis* derived antigens used to analyse the anti-*P. gingivalis* antibody response.

Two mechanisms through which *P. gingivalis* may orchestrate the humoral response and stimulate autoimmunity have been proposed: i.) citrullination by *P. gingivalis* peptidyl arginine deiminase (PPAD); and ii.) autocitrullination of its own PAD (Rosenstein *et al.*, 2004).

***P. gingivalis* Peptidyl Arginine Deiminase and Citrullination: Cross talk between the pathobiology of RA and PD**

Bacterial Peptidyl Arginine Deiminase (PAD) also known as *P. gingivalis* peptidyl arginine deiminase (PPAD) is able to citrullinate free arginine as well as C-terminal arginine residues without requiring calcium. In humans, 5 types of PAD enzymes have been identified (PAD-1, PAD-2, PAD-3, PAD-4 and PAD-6) with different functions and they are distributed in various locations (Valesini *et al.*, 2015). They catalyse tissue citrullination as part of the normal physiologic process. Unlike PPAD, the activity of human PAD is dependant on the presence of calcium. To date, *P. gingivalis* is the only known prokaryote that can produce PAD (McGraw *et al.*, 1999).

Other virulent factors of *P. gingivalis*, specifically Gingipains (a group of trypsin-like cysteine proteinases) have been deemed to play a part in citrullination (Potempa *et al.*, 2003). Gingipain works together with PPAD by supplying arginine-containing residues to facilitate citrullination (Wegner *et al.*, 2010). Besides, gingipains have been shown to increase intracellular calcium concentrations by cleavage of proteinase-activated receptor 2, a G protein-coupled receptor found on the neutrophil surface, which may in turn promote human PAD activation (Lourbakos *et al.*, 1998).

In their animal study, Maresz *et al.* (2013) demonstrated that collagen-induced arthritis was aggravated in mice infected with live *P. gingivalis*, therefore suggesting a mechanistic link via citrullination between *P. gingivalis* infection and PPAD expression with RA exacerbation. Therefore, PPAD expression was a possible trigger of a pathogenic autoimmune response in RA.

Gully *et al.* (2014) supported the aforementioned argument by associating PPAD deficient strains of *P. gingivalis* with reduced amounts of periodontal bone loss, less severe experimental arthritis and lower levels of anti-citrullinated protein antibody. However, it may be well that the lower prevalence of periodontitis was the cause for the reduction in the prevalence of RA and not necessarily the PPAD expression per se.

P. gingivalis is capable of citrullinating both its own and host proteins. Although PPAD has a preference for C-terminal arginine residues, some studies have however recognized this enzyme's ability to self-deiminate, also known as auto-citrullination. Rodriguez *et al.* (2009) found a loss of arginine residues from freshly purified PAD at the end of 21 days and the presence of citrulline residues was found to be directly proportional with the disappearance of arginine from the protein. However, despite the evidence, they did not denote whether internal or C-terminal arginine residues had been citrullinated. Quirke *et al.* (2014) confirmed autocitrullination of PPAD by mass spectrometry and matched the figure with the presence of citrulline detected internally. Their study reported that 38% of RA patients with PD had peptidyl citrulline-specific antibodies to PPAD, as opposed to 2% of PD patients. A specific immune response to autocitrullinated PPAD suggests that PPAD could be an antigenic target. Hence, in susceptible individuals, PPAD may trigger an immunological response to citrullinated proteins and thus lead to the breakdown of tolerance.

In contrast to this postulate, König *et al.* (2015) explained that PPAD autocitrullination was not contributory to the interrelationship between RA and PD because autocitrullination was merely an in vitro cloning feature which would not occur naturally. In their experiment, they noticed anti-PPAD antibodies were reduced in RA patients with PD. Their finding was however dis-

puted again by Quirke *et al.* (2015). The same experiment was repeated by Quirke and colleagues (2015) in RA and OA (osteoarthritis) patients and they found higher anti-PPAD antibody response in sera samples of RA patients compared to OA patients thus rebutting the findings of König and colleagues (44). Such remarkable discrepancy between the findings may be due to differences in patient cohorts and ELISA (Enzyme-Linked ImmunoSorbent Assay) methodology. Similarly, another two case-control studies looking at the association between anti-PPAD antibodies and the development of RA in a pre-RA cohort also reported contradictory results (Fisher *et al.*, 2015; Johansson *et al.*, 2016). Johansson and colleagues detected antibodies against *P. gingivalis* (anti-CCP3, a synthetic citrullinated peptide derived from PPAD) 8 years before the onset of symptoms of RA irrespective of smoking habit (Johansson *et al.*, 2016). In stark contrast of Johansson *et al.*, Fisher and colleagues found no association between anti-PPAD and risk of RA or with ACPA, suggesting that factors other than *P. gingivalis* infection may contribute to the link between PD and RA (Fisher *et al.*, 2015). However, the sample size of Fisher *et al.* was smaller and a portion of the RA subjects included had unconfirmed diagnosis which may account for the inconsistencies in findings from these two studies. Further investigations therefore need to be performed to assess the actual contribution, if any, of autocitrullination in the RA-PD link.

In a subsequent study, König *et al.* (2016) showed that *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) was also able to induce hypercitrullination in neutrophils. This was postulated to be caused by the leukotoxin A (LtxA) produced by *A. actinomycetemcomitans* which can dysregulate citrullinating enzymes in neutrophils and also cause changes in neutrophil morphology which enables it to release its hypercitrullinated cargo (Figure 3). However a subsequent study done on a larger sample population showed no difference in the antibody to LtxA between RA and other forms of arthritis (Volkov *et al.*, 2018).

***P. gingivalis*' role in modulation of gut microbiota and gut immunity**

Based on the disputes to the hypothesis linking PPAD with RA, other links between *P. gingivalis* and RA have been explored. Animal studies have shown that collagen-induced arthritis has been mediated through the introduction of oral antigens in the setting of mucosal barrier dysfunction (Wu *et al.*, 2013) and also an elevation in serum IL-17 levels (de Aquino *et al.*, 2014). A recent mice study by Sato *et al.* (2017) reported that oral administration of *P. gingivalis* may have an effect on RA through significant changes that takes place in the gut microbiota. It was found that the population belonging to the phylum Bacteroidetes was significantly elevated in *P. gingivalis*-administered mice which was concomitant

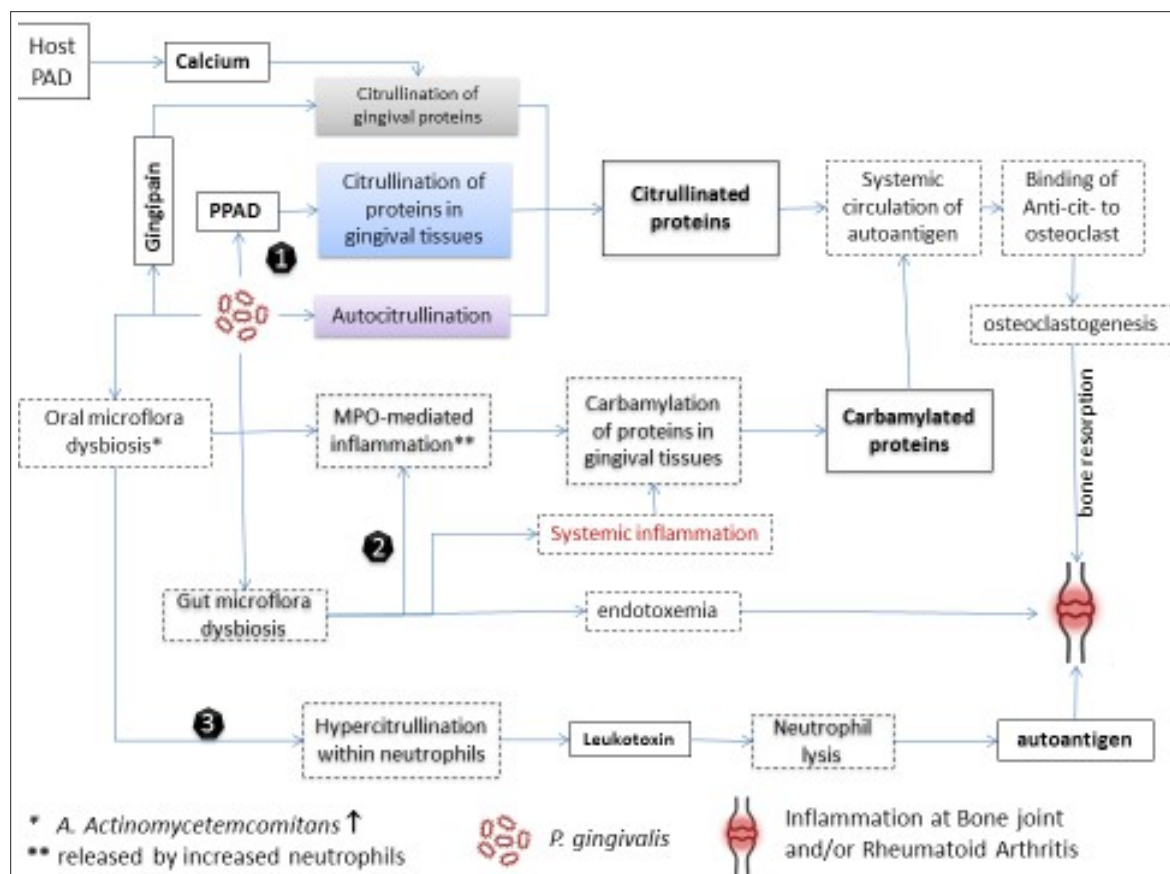


Figure 3. Possible mechanisms on how PD aggravates RA.

1. The role of *P. gingivalis* in contributing to the formation of autoantigens either directly through protein citrullination or indirectly through inflammation-mediated carbamylation.
2. *P. gingivalis* as a keystone pathogen in causing alterations to the gut microflora and potentially leading to endotoxemia and the persistence of low-grade systemic inflammation which may exacerbate the inflammatory response within the joint.
3. Extracellular release of autoantigen following neutrophil apoptosis as a result of *A. actinomycetemcomitans*-induced hypercitrullination.

with increases in insulin resistance and systemic inflammation (Arimatsu *et al.*, 2014). In these mice, blood endotoxin levels tended to be higher, whereas gene expression of *tjp-1* and *occludin*, which are involved in intestinal permeability, were downregulated. This dysbiosis in the gut microbiota then causes an up-regulation of TH 17 cell proportions and IL-17 sera levels but there was however no increase in anti-citrullinated protein antibody levels. This therefore shows an alternative mechanism by which *P. gingivalis* may shift the gut immune system towards TH17 dominance and ultimately involved in the pathogenesis of RA (Gaffen, 2009). However further studies need to be done to confirm this link.

Future microbial studies

Current studies looking at periodontal bacteria induced inflammation linking PD and RA have focused on *P. gingivalis*. These studies have mainly utilized serological methods in its methodology. Thus far few studies have assessed the subgingival microbiota in patients with RA using next generation

sequencing (Beyer *et al.*, 2018; Lopez-Oliva *et al.*, 2018; Mikuls *et al.*, 2018). Scher and colleagues (2012) looked into the oral microbiota association with RA-PD using pyro-sequencing. The investigators demonstrated that subgingival microbiota profile in patients with new-onset RA was similar to that in patients with chronic RA and healthy subjects whose PD was of comparable severity. However they noted that *Leptotrichia* and *Prevotella* were found in abundance in newly diagnosed untreated RA. In another recent study (Lopez-Oliva *et al.*, 2018), *Cryptobacterium curtum* was identified in the RA group and this bacteria has been shown to be capable of producing large amounts of citrulline. Mikuls *et al.* (2018) failed to identify bacterial fingerprints specific to RA but suggested that other factors impacting oral health status may have influenced oral microbiome composition. Similarly, Beyer and colleagues (2018) concluded that the oral microbiome composition depended largely on active or remission status of RA patients whereby the remission group which had more current smokers and lower oral health status had higher proportions of perio-pathogenic taxa.

In recent times, newer technologies have emerged whereby whole-genome shotgun (WGS) sequencing of all bacterial genomes in a metagenomic sample at high coverage can be performed using Illumina HiSeq technology, rather than just sequencing the 16S rRNA genes that was previously done using pyro-sequencing. Unlike previous 16S-based approach, this approach offers a more global view of the microbial community, studies the full set of genes and metabolic pathways in the community, provides functional variations between different microbial communities, allows better assessment of phylogenetic diversity and at the same time has the potential to discover new genes. Results from metagenomic studies performed on chronic periodontitis subjects have demonstrated strong correlation between community structure and disease status with diseased samples having a higher microbial diversity (Wang *et al.*, 2013). Future metagenomics studies on RA subjects with PD should be performed to better assess the subgingival microbial diversity in these patients and perhaps may identify other links in the RA-PD association.

Carbamylation

Although the role of citrullination in the pathogenesis of RA has long been established, it is only in recent years that a different form of post-translational modification of proteins, known as carbamylation has emerged as a potential trigger factor for RA. Unlike citrullination, carbamylation is a non-enzymatic chemical reaction with cyanate that converts lysine residues to homocitrulline. In healthy states, cyanate is in equilibrium with urea and its concentration is too low to permit excessive carbamylation to occur. However, Wang and colleagues (2007) proved that a myeloperoxidase (MPO)-mediated pathway for protein carbamylation could take place under inflammatory conditions. MPO is released from neutrophils during inflammation and increases the concentration of cyanate by converting thiocyanate to cyanate. The new role of carbamylation in the pathogenesis of RA was discovered following an animal experiment on mouse models (Mydel *et al.*, 2010). In the experiment, carbamylated peptides in immunized mice triggered T & B cell activation and antibody production. The immunized mice also showed a higher susceptibility to a more severe form of arthritis than the non-immunized mice.

In the pathogenesis of periodontitis, neutrophils form the first line of defense through intracellular and extracellular killing mechanisms. Other than phagocytosis, neutrophils can also undergo pre-programmed cell death by releasing neutrophil extracellular traps (NETs) to control infection. Myeloperoxidase is required for NET formation so following the discovery of NET in gingival purulent discharge, the role of myeloperoxidase in periodontal health has been renewed (White *et al.*, 2016). Therefore, the inflamed periodontal tissues may serve as a potential site of MPO-driven protein carbamylation.

MPO-mediated pathway coupled with citrullination induced by *P. gingivalis* in periodontitis may possibly be responsible for prompting the breakdown of immune tolerance to auto-antigens and progressing to RA in individuals with genetic vulnerability (Bright *et al.*, 2015).

About 16-30% of anti-citrullinated peptide antibodies negative patients were tested positive for anti-CarP antibody (Brink *et al.*, 2015; Shi *et al.*, 2011; Stoop *et al.*, 2014). On the other hand, several studies have shown that anti-carbamylated peptide (anti-CarP) antibodies are present years before the clinical onset of RA and are positively associated with radiological damage of the joints and disease progression. A recent meta-analysis by Li *et al.* (2016) concluded that anti-CarP has a moderate diagnostic value in RA. This information is clinically useful to identify asymptomatic pre-RA patients and enable early intervention for better prognosis. A recent study has provided evidence of the presence of carbamylated proteins in inflamed periodontal tissues (Bright *et al.*, 2018). Furthermore elevated serum levels of carbamylated proteins has been shown in patients with RA and PD (Kaneko *et al.*, 2018). Further research is necessary to confirm and ascertain the inter-relationship between carbamylation and the development of both RA and PD. The infectious links associating PD with RA are depicted in Figure 3.

Common Tissue Destruction Pathways

Apart from the genetic and infectious links between RA and PD, some studies looked into their resemblances in the tissue destruction pathway. In Golub's "two-hit" model, the local inflammatory responses in periodontium (1st hit) and synovium (2nd hit) stimulate systemic inflammatory response through the increase of inflammatory mediators in the circulation and further exacerbate each other (Golub *et al.*, 2006). While the 'two-hit' model is a favourite concept used to explain the link between RA and PD, another hypothesis that has been proposed is that at least one common underlying dysregulation of the inflammatory pathway between RA and PD may lie within the RANKL (receptor activator of nuclear factor kappa-B ligand)/OPG/TRAIL axis (Bartold *et al.*, 2005). A decrease in osteoprotegerin (OPG) expression leads to vascular damage whereas an increase in RANKL and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) levels may result in the activation of osteoclasts and consequently bone resorption.

RANKL is a cell-surface protein and a member of the tumour necrosis factor (TNF) superfamily. It can be secreted by several cells including osteoblasts, stromal cells and dendritic cells as well as activated T and B cells. It binds to its receptor, known as RANK on osteoclast precursor cells and promotes the maturation of osteoclasts which in turn causes bone resorption. OPG, on the contrary, is a soluble cytokine receptor that acts as a natural inhibitor of RANKL. Therefore, no bone destruction will transpire when OPG binds to RANK

(Simonet *et al.*, 1997; Wang *et al.*, 2003). Arg-gingipain of *P. gingivalis* has also been shown to increase the RANKL/OPG ratio in gingival fibroblast and periodontal ligament cells and subsequently enhanced osteoclastogenesis (Belibasakis *et al.*, 2007).

There has been growing literature pertaining to the role of TRAIL in tissue destruction pathway. There are five types of receptors for TRAIL, two death receptors with death domains and three decoy receptors without the death domains. Programmed cell death is activated when TRAIL binds to the death receptors whilst apoptosis is inhibited when the decoy receptors bind to TRAIL (Lucas *et al.*, 2010). It is believed that in both RA and PD, either a reduced TRAIL death receptor expression or a raised expression of TRAIL decoy receptor may be the reason large numbers of chronic inflammatory cells persist in the diseased tissues (Agnihotri and Gaur, 2014; Lucas *et al.*, 2010).

The currently available literature focuses on either the ratio of RANKL/OPG in periodontal lesions or the expression of TRAIL in RA tissues but none on the comparison of the RANKL/OPG/TRAIL axis in two disease entities. Perhaps, future studies need to investigate the levels of RANKL, OPG and TRAIL simultaneously in both RA and PD lesions in order to validate the aforementioned hypothesis.

Conclusion

In vivo and *in vitro* research over the past few decades have shed some light on the biological mechanisms connecting PD to RA but the exact pathogenesis is still too complex to unify the previously proposed mechanisms. Given the fact that both RA and PD are complex in nature, it may take years or even decades to find the answer. It is prudent to understand the potential mechanisms linking PD to RA as the patho-biological similarities borne between both diseases suggest that the common/combined treatment of RA and PD may have beneficial effects on both diseases.

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