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Periodontal disease and periodontal bacteria as triggers for rheumatoid arthritis

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Abstract

There is an epidemiological association between periodontitis and rheumatoid arthritis (RA), hypothesised to lead to enhanced generation of RA-related autoantibodies, which can be detected years before the onset of RA symptoms. Periodontitis is a common dysbiotic disease; tissue damage occurs because the immune system fails to limit both the resident microbial community and the associated local immune response. Certain periodontal bacteria, including *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, may contribute to RA-autoantibody production through direct post-translational modification of proteins or, indirectly, by influencing neutrophil-mediated neo-epitope generation. Oral bacteria that invade the blood may also contribute to chronic inflammatory responses and generation of autoantibodies. The putative association between periodontitis and the development of RA raises the potential of finding novel predictive markers of disease and disease progression, and for periodontitis treatment to be included in the future as an adjunct to conventional RA immunotherapy or as part of a preventive strategy.

Keywords

Rheumatoid arthritis; Periodontitis; Autoantibody; Subgingival microbiome; *Porphyromonas gingivalis*

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease that manifests as a chronic polyarthritis. There is increasing evidence that the initiating events that result in the generation of RA-related autoantibodies, which can be detected years before the onset of RA symptoms, occur at mucosal sites distant to the joints [1-5]. Inflammatory processes in response to environmental triggers, including infections, in the lungs and the mouth have been strongly implicated and, recently, also in the gastrointestinal and genitourinary tracts [4-7]. This review concentrates on the contribution of oral disease, specifically periodontal disease, and oral bacteria to the development of RA.

Periodontal diseases are common oral inflammatory conditions that occur in response to bacterial plaque biofilms, causing damage to the gingivae (gums), periodontal ligament and alveolar bone, all of which form the supporting tissues of the teeth (Figure). Severe periodontitis occurs in 2-20% of most adult populations, affecting 300 million people worldwide [8]. In the UK, 3-4 million currently suffer from advanced periodontitis at a cost of £2 billion/year to the National Health Service (NHS). Associations have emerged between periodontitis and a growing list of chronic conditions including atherosclerosis, diabetes and RA [9-11].

The links between rheumatoid arthritis and periodontal disease.

RA and periodontitis display some pathogenic similarities, such as the host immune response leading to soft tissue inflammation with subsequent hard tissue destruction, and certain risk factors, including smoking and excess weight or obesity, although some studies only show associations at specific stages of disease aetiology [12-17]. The significant RA risk attributed to the shared epitope HLA-DRβ1 (SE) is well established [18], but associations of specific human leukocyte antigen (HLA) molecules with chronic periodontitis are unclear.

Multiple studies have shown an epidemiological association between periodontitis and RA and these have been reviewed recently [6, 19, 20]. Inconsistent diagnosis of periodontal disease may have led to an alternative conclusion in some studies [6, 21]. However, a systematic review and meta-analysis confirmed an elevated risk of periodontitis in RA patients compared with healthy controls [19]. Analyses of detailed clinical data have revealed significantly raised indicators of the severity of periodontal disease (mean probing depth; bleeding on probing, BOP; absolute clinical attachment loss, CAL; tooth loss) in people with RA compared to those without [19, 22].

Understanding the common mechanisms that underlie periodontitis and RA could present new possibilities for the treatment and prevention of RA. The link between these conditions was further highlighted in a recent study where patients with periodontitis and arthralgia who later developed RA had higher levels of disease activity and were more likely to receive methotrexate at RA diagnosis compared to patients without periodontitis [23].

Periodontal diseases

Periodontal diseases are complex polymicrobial conditions resulting from an imbalance between the resident subgingival microbial communities, which grow as biofilms adhered to the tooth and tissue surfaces, and host responses to them. In these dysbiotic diseases, damage to the supporting tissues of the teeth occurs because the immune system fails to control both the microbial communities and the local host immune response to them [24].

The term, periodontal disease, describes a spectrum of inflammatory conditions. Gingivitis is an inflammatory response to the accumulation of dental plaque at the gingival margin (Figure). It is reversible and can be eradicated by maintaining good oral hygiene. Conversely, the damage associated with periodontitis is irreversible; chronic inflammation within the supporting tissues of the teeth (Figure) and the consequent tissue destruction and

gingival epithelial migration cause progressive attachment loss and bone loss, characterised by periodontal pocket formation and/or gingival recession [25].

The most common form of periodontitis is chronic adult periodontitis, which is assessed as mild, moderate or severe/advanced depending on the extent of BOP, periodontal pocket formation, radiographic bone loss and CAL [25]. Aggressive periodontitis is a less common, severe form of the disease which often occurs in people under 25 years. It may be localised to certain teeth or generalised, and can be associated with a more sparse plaque than that observed in chronic adult periodontitis. Two RA-associated genes that function in Interferon-β (IFN-β) signalling were highlighted in cases of aggressive periodontitis as shared susceptibility factors, but the aetiologies of aggressive and chronic periodontitis differ and the genetic contribution may be lesser in chronic periodontitis [26, 27].

The microbiology of periodontal diseases

Periodontal pockets can reach a probing depth of up to 12mm; this stagnant and anaerobic site may harbour up to 10^8 diverse bacteria [28]. More than 700 bacterial species have been identified from the human mouth; only about 60% of these can currently be cultured in the laboratory [29], so nucleic acid based methodologies are essential to understand the entirety of the health and disease-associated microbiota. It is important to recognise that periodontitis is a polymicrobial infection caused by co-operating consortia of organisms [30]. Organisms associated with severe periodontitis are often also isolated from healthy sites, albeit in low numbers; pathogenic communities arise from the normal microbiota through processes of selection in response to local environmental pressures that are associated with inflammation and bleeding, and through the failure of the host responses to control the subgingival microbiota [31, 32]. As periodontitis develops, there is a transition from plaque

dominated by Gram-positive facultatively anaerobic species, to communities that are dominated by obligately anaerobic, proteolytic Gram-negative rods and spirochaetes [32].

Many organisms increase in abundance with the development of periodontitis, and newly described potential pathogens are emerging [32, 33]. *Porphyromonas gingivalis* may function as a "keystone pathogen" in chronic periodontitis, playing a disproportionately important role by depressing and deregulating local immune responses, increasing the virulence of the whole community and promoting the dysbiosis that is characteristic of periodontitis [30]. It is in turn dependent on the activities of accompanying accessory organisms (e.g. *Streptococcus gordonii*) to express its full pathogenicity [34]. *Aggregatibacter actinomycetemcomitans* is associated with localised aggressive periodontitis (LAP), in which it may function as a keystone pathogen [35, 36]; a combination of *A. actinomycetemcomitans*, *Filifactor alocis* and *Streptococcus parasanguinis* was highly predictive of bone loss in individuals susceptible to LAP [35]. Viruses are only rarely considered, but they may also play a role in development of periodontitis [37].

Oral host-microbe homeostasis is maintained by the constant control of the microbial burden and protection mediated by inflammatory and immune defences. Periodontal pathogens manipulate, dysregulate and subvert these defence mechanisms, disabling protective mechanisms and disrupting control of the microbiota. Inflammophilic species, such as *P. gingivalis*, dysregulate processes to drive inflammation and elicit tissue damage, yielding a supply of nutrients to support their survival.

The roles of host defences

Both innate and adaptive immune functions are important to the development of periodontitis. It is beyond the scope of this review to discuss all immune contributions to the disease in detail but they have been extensively reviewed recently [38, 39].

The chronic nature of inflammation in periodontitis allows for substantial lymphocyte involvement, including significant B and CD4+ T cell infiltration into gingival tissues and increased expression of Th1 and Th17 cytokines and receptor activator of nuclear factor κ-B ligand (RANKL). RANKL stimulates osteoclastogenesis and subsequent resorption of alveolar bone [39, 40]. Expression of genes encoding IL-1β, IL-6, IL-21 (supporting Th17 differentiation) have been detected in diseased gingival tissue, in addition to IL-23-producing macrophages that amplify Th17 responses [40]. Increases in Th17 cells in the synovium of RA joints have also been reported [39]. In periodontitis, elevated IL-17 levels may perpetuate phagocyte recruitment and induce osteoclastic differentiation of monocytes [38, 40, 41]. A counterbalance to Th1 and Th17 CD4 T cell activity may be provided by CD4 TReg cells, by secretion of immunosuppressive IL-10 and TGF-β, but evidence for the role of IL-10 in periodontal health/disease is equivocal [40].

Innate immunity is involved from the early stages of periodontal disease. Some periodontal bacteria dysregulate the functions of Toll-like receptors (TLRs) expressed by cells in gingival tissues, leading to tissue damage and periodontal disease pathogenesis [24]. Complement is vital, both early in the development of dysbiosis and in driving the inflammatory destruction of periodontal tissue, and the alternative pathway of complement activation predominates in periodontitis [42]. Some bacteria (e.g. *P. gingivalis, F. alocis*, *Prevotella intermedia, Treponema denticola*, *Tannerella forsythia*) manipulate the complement system, e.g. through binding and/or proteolytic cleavage of endogenous inhibitors, C3 convertase or C5 while allowing release of anaphylatoxin C5a. These strategies allow bacteria to evade complement-mediated microbicidal activities, while promoting inflammation and neutrophil recruitment to the periodontal pocket.

Neutrophils are of primary importance in the maintenance of gingival homeostasis [43]. In health, resident bacteria stimulate gingival epithelial cells to establish a CXCL-8 chemotactic

gradient and upregulate expression of the neutrophil chemotactic receptor, CXCR-2, thereby promoting neutrophil homing to periodontal tissue and their formation into a protective barrier between the biofilm and host [44]. Neutrophils account for 90% of the leucocytes in gingival crevicular fluid (GCF) and their concentration increases 15-fold in periodontally diseased sites [45]. Their fundamental protective role is illustrated by the often severe periodontitis associated with iatrogenic neutropenia and with inherited dysfunctions in neutrophil effector functions, e.g. Chediak-Higashi and Papillon Lefevre syndromes. Impaired neutrophil chemotaxis has been reported in periodontitis and periodontal pathogens employ various strategies to disrupt neutrophil chemotaxis and/or function [39, 42, 46].

The neutrophil antimicrobial arsenal includes the generation of reactive oxygen species (ROS), the release of granule contents which include matrix metalloproteinase 8, gelatinases, myeloperoxidase (MPO), neutrophil serine proteases and antimicrobial peptides such as α-defensins and hCAP-18 (the LL-37 precursor). Neutrophils generate Neutrophil Extracellular Traps (NETs), decondensed webs of chromatin that are decorated with antimicrobial proteins derived from neutrophil granules. A widely held view is that NET generation is facilitated by NADPH oxidase, neutrophil elastase and peptidyl arginine deiminase 4 (PAD4); PAD4 converts positively charged arginine residues within histone proteins into neutral citrulline, thereby disrupting electrostatic interactions and inducing chromatin decondensation [46, 47]. Increased NET production, or impeded NET clearance, may contribute to inflammatory responses as NETs provide an extracellular reservoir of inflammatory components, such as LL-37, bacterial components, ds-DNA and hypercitrullinated proteins. PAD4-/- mice are more susceptible to bacterial infections and NETs have been detected in the GCF from periodontal disease sites in abundance [47].

In addition to their importance in periodontal diseases, neutrophils and periodontal bacteria have been implicated in mechanisms that increase the generation of autoantibodies that are important in the development of RA.

Autoantibodies in RA and periodontal disease.

The importance in RA of autoantibodies against proteins that have undergone post-translational modification (PTM) has been extensively reviewed recently [1-3]. Some of these antibodies have also been observed in periodontal tissues and disease [26, 48].

Citrullination, a PTM of arginine, is involved in the formation of hair, skin, myelin sheaths, in NET formation and inflammation, and in cell death [1]. It is mediated by PAD enzymes, of which there are five in humans [49]. Citrullination alters tertiary protein structure and function and may expose previously hidden immune epitopes [50]. Neutrophils are enriched for PADs and calcium-associated hyper-activation of neutrophil PADs leads to hypercitrullination of proteins [49]. However, there is an active debate concerning the methods employed to study NETosis, the roles of PADs and the routes to protein hypercitrullination, with the proposal that exposure of neutrophils to bacterial pore-forming toxins, complement membrane attack complex (MAC) or perforin leads to generation of NET-like structures and a process of leukotoxic hypercitrullination [49].

Serum anti-citrullinated protein antibodies (ACPAs) are present in 70% of RA patients; they are associated with RA progression and may be detectable up to 10 years before the onset of clinical disease [48]. Citrullinated proteins have been detected in periodontal tissues [51, 52] and there are significant associations between ACPA seropositivity and periodontal disease [53, 54]. Therefore, a popular hypothesis is that in genetically susceptible individuals, citrullination associated with periodontitis may cause a localised oral mucosal immune response, which can lead to a systemic ACPA response, followed by synovial inflammation

and the onset of RA [55]. However, Konig *et al.* have challenged the hypothesised central role for autoantibodies against citrullinated proteins in the loss of tolerance in RA development, asserting the importance of antibodies against native unmodified proteins as the driving force behind loss of immune tolerance, preceding development of ACPAs [56].

Carbamylation is a non-enzymatic PTM in which cyanate binds to the primary amine of lysine and forms carbamyl groups, generating peptidyl-homocitrulline against which autoantibodies (anti-CarP) are generated [2]. Neutrophil MPO can enhance protein carbamylation by promoting generation of cyanate from thiocyanate [57]. Like citrullination, carbamylation may affect protein function, e.g. carbamylation of immunoglobulin G (IgG) can inhibit classical complement pathway activation [58]. Anti-CarP have been detected in ACPA-negative and ACPA-positive pre-RA and established RA patients [59, 60], and were predictive of the development of RA independently of anti–CCP2 (citrullinated cyclic peptide 2) antibodies [61]. In ACPA-negative patients, anti-CarP antibodies are predictive of a more severe RA disease course [62]. However, there were no significant associations between anti-CarP and RA genetic risk factors or smoking, suggesting anti-CarP antibody formation occurs via different biological mechanisms to ACPA formation [63]. A recent study detected a weak association between ACPA seropositivity and periodontitis but there was none between periodontitis and anti-carP seropositivity [53], although carbamylated proteins were detected in inflamed gingival tissues [48] and MPO was elevated in periodontitis [64, 65].

Antibodies against proteins modified with malondialdehyde-acetaldehyde adducts (MAA) were increased in established RA patients and were associated with ACPA and RF detection [66]. MAA are generated when lipid peroxidation by ROS (produced during oxidative stress and released from neutrophils) forms highly reactive malondialdehyde and acetaldehyde molecules, which modify lysine residues of proteins to generate stable MAA [67].

Preliminary data indicate injection of mice with *P. gingivalis* could increase production of MAA antibodies [68].

Porphyromonas gingivalis, RA and autoantibody production

P. gingivalis expresses several virulence factors, such as fimbriae, lipopolysaccharide, capsular polysaccharide and cysteine proteases (gingipains). These collectively contribute to its ability to colonise, invade and damage host tissues, and also to degrade and dysregulate local immune responses [43]. The arginine-specific (RgpA and RgpB) and lysine-specific (Kgp) gingipains are crucial for *P. gingivalis* survival and growth in the anaerobic periodontal pocket [69] and they are fundamental to its ability to manipulate host immune responses [70, 71].

P. gingivalis also produces a peptidyl-arginine deiminase (PPAD) capable of citrullinating host and bacterial proteins, but which has no sequence homology with human PADs [72]. Unlike human PADs, PPAD preferentially citrullinates terminal arginines and also free arginine, and works best at the slightly alkaline pH that is optimal for *P. gingivalis* growth [72, 73]. Rgp gingipains cleave polypeptide chains at internal arginine residues, generating peptides with terminal arginines that are susceptible to PPAD citrullination [74]. PPAD activity has been detected in GCF from periodontitis patients and at lower levels in healthy controls [75]. It is capable of auto-citrullinating some of its 18 arginine residues [76], although there is evidence that anti-PPAD antibodies are not directed against the citrullinated form of PPAD and that in humans, PPAD is not modified in this manner [77]. PPAD enhances cell invasion by *P. gingivalis* [78] and citrullinates host defence components, such as complement and LL-37, with consequent loss of function [79, 80]. Human fibrinogen and α-enolase, two of the proteins targeted by ACPAs in RA [74], are also PPAD substrates and

255 antibodies against auto-citrullinated *P. gingivalis* enolase cross react with human α-enolase
256 autoantibodies [48].

257 Animal model studies support the hypothesis that *P. gingivalis* is important in the aetiology of
258 RA. *P. gingivalis* expressing PPAD accelerated progression and enhanced severity of
259 collagen-induced arthritis in mice and was associated with higher levels of citrullinated
260 proteins at diseased sites [81]. Exposure to *P. gingivalis* in mice expressing human
261 HLA-DRβ1 impaired resistance to the development of arthritis and induced autoimmune
262 arthritis, and generated increased Th17 cell frequency, systemic cytokine activity and ACPA;
263 both PPAD and the HLA-DR1 restriction were needed to drive ACPA generation [82].

264 Epidemiological studies of the associations between *P. gingivalis*, PPAD or Rgp and RA
265 (including pre-RA) have been equivocal. DNA from *P. gingivalis* was detected in synovial
266 fluid of RA patients more often than in controls [83] and more often in the GCF of RA patients
267 compared with controls [75]. Although one study found no increase in anti-RgpB antibodies
268 in RA sera [76], another found that anti-RgpB antibody levels were significantly elevated in
269 ACPA-positive RA patients compared with ACPA-negative, and the significant association
270 between anti-RgpB IgG and RA was stronger than that between smoking and RA [84].
271 There are conflicting data and opinions regarding the relationship of PPAD with RA.
272 Elevated PPAD activity in GCF was not clearly associated with RA even though *P. gingivalis*
273 detection in GCF was [75]. While one study found anti-PPAD antibodies were elevated in
274 RA sera compared with sera from controls [76], another found anti-PPAD antibodies did not
275 correlate with ACPA levels or RA disease activity and levels were decreased in RA patients
276 with PD [77]. Methodological differences have been suggested to account for this
277 discrepancy [85]. A recent study of RA patients on disease-modifying anti-rheumatic drug
278 (DMARD) therapy, found a correlation between anti-PPAD IgG and anti-CCP IgG, both of
279 which were significantly increased in the RA group compared with controls [86]. RA patients

280 treated with biological DMARDs who had low anti-PPAD IgG titres showed a significantly
281 greater decrease in RA disease activity score compared with patients with high anti-PPAD
282 IgG titres, indicating that serum IgG anti-PPAD may be useful as a predictive biomarker for
283 response to RA therapy [87].

284 Most studies have focused on patients with established RA; to better understand
285 pathogenesis and develop therapies it is important to also investigate individuals at risk for
286 the development of RA. An increased concentration of anti-*P. gingivalis* antibodies has
287 been reported in individuals at genetic risk of developing RA (some also had RA-related
288 autoantibodies) [88]. Furthermore, higher anti-RgpB IgG levels were found in the blood of
289 pre-RA and established RA individuals compared with healthy controls; while ACPA levels
290 increased with time, anti-RgpB antibody levels did not and they decreased following
291 diagnosis [89]. In contrast, no association between anti-RgpB and pre-RA was found in a
292 different study of a Southern European cohort [14]. Importantly, these studies did not
293 evaluate clinical periodontal status alongside *P. gingivalis* antibody levels. In a recent study
294 of an early inflammatory arthritis cohort, periodontitis, but not the subgingival presence of *P.*
295 *gingivalis*, was more enriched in patients who later progressed to classifiable RA [23].
296 Similarly, De Smit *et al* concluded that, while there was evidence that periodontal disease
297 may precede symptomatic RA, there was insufficient evidence to confirm a role specifically
298 for *P. gingivalis* in disease progression [90].

299 Thus, while the link between periodontitis and RA is established, the specific roles of *P.*
300 *gingivalis* or PPAD are less clear. This could partly be due to strain-to-strain differences,
301 although it is not yet known if there is any difference in the activity of PPAD from different *P.*
302 *gingivalis* strains/genotypes. Five distinct *rgpB* genotypes have been found in clinical *P.*
303 *gingivalis* isolates and the activity of the expressed gingipains would impact on that of PPAD
304 [91]. The activities of other bacteria in the subgingival community may also be influential;

305 although *P. gingivalis* is a “keystone pathogen” that increases the risk of periodontitis, it
306 depends upon the activities of other members of the microbiota to colonise, grow, invade
307 epithelial cells and express its full virulence [34].

308 **Multiple mechanisms may be important**

309 Periodontitis is a complex disease, mediated by consortia of co-operating bacteria and the
310 host responses to them. It is, therefore, logical to widen consideration of the influence of the
311 microbiota beyond that of a single, albeit important, bacterium. For example, the leukotoxin
312 produced by *A. actinomycetemcomitans* has been implicated in inducing leukotoxic
313 hypercitrullination, and exposure to *A. actinomycetemcomitans* was associated with ACPA
314 and rheumatoid factor (RF) [92]. The subgingival microbiota of periodontitis is enriched for
315 obligately anaerobic proteolytic bacteria [32] and they may contribute alongside *P. gingivalis*
316 to the enzymatic cleavage of host proteins, particularly components of the extracellular matrix,
317 and enhanced generation of neo-epitopes [93, 94]. Using 16S rRNA sequence analysis of
318 the entire subgingival microbiome, Scher *et al.* found that the microbiome of RA patients was
319 similar to healthy subjects with similar periodontal status, but, specific *Prevotella* and
320 *Leptotrichia* operational taxonomic units (OTUs) were only found in new-onset RA patients,
321 and *Anaeroglobus geminatus* was correlated with the presence of ACPA and RF, and with
322 periodontitis [95]. Another large-scale study using metagenomic shotgun sequencing
323 identified compositional and functional alterations in RA-associated oral microbiomes, which
324 were partly resolved by DMARD treatment; thus, this big data approach suggests that
325 microbiome composition could be important in prognosis and diagnosis of RA [96].

326 Neutrophils are key players in both RA and periodontitis. They can promote autoantibody
327 production by multiple routes, all of which may be important in RA, and they also contribute to
328 the immune dysregulation and tissue damage associated with periodontitis. Interference

with the normal functions of neutrophils is an important pathogenic strategy employed by many periodontal bacteria and some of these may in turn promote neutrophil mediated autoantibody production; e.g. the pore-forming leukotoxin of *A. actinomycetemcomitans* [49]; *F. alocis* promotion of neutrophil degranulation [97]; *P. gingivalis*, *A. actinomycetemcomitans* and *F. nucleatum* triggering the release of NETs [47].

While local responses are important, systemic influences on blood should be considered. Peripheral blood neutrophils in patients with inflammatory diseases such as periodontitis and RA have been reported to display an activated phenotype with hyperactive respiratory burst responses and, in RA, increased NETosis [47, 98, 99]. Oral bacteria regularly gain access to the blood and have been detected at distant sites such as the heart and also in synovial tissue samples [100]. Pretorius *et al*. have proposed that an aberrant blood microbiome may play a significant role in the aetiology of RA [101] and other systemic diseases that have been linked to periodontitis [102]. Microscopic analysis of blood from periodontitis patients revealed bacteria associated with erythrocytes at a much higher prevalence than seen in blood from healthy controls [102]. In this analysis, bacteria that gain ingress into the blood may remain dormant, most likely because they are deprived of essential iron; dormant bacteria are associated with circulating cells including erythrocytes and in this state they may constitute a persistent supply of inflammatory molecules including lipopolysaccharide. The authors propose this may be a unifying principle underlying the links between inflammatory diseases such as periodontitis and a range of systemic diseases including RA.

Practice points:

The association between RA and periodontitis indicates the potential benefits of the closer integration of medical and dental care:

- RA patients have an increased prevalence of periodontal disease and therefore should be encouraged to have regular dental assessments
- Periodontal disease may be associated with increased RA disease activity; if periodontal disease is identified in a patient with RA it should be managed by a dentist
- Individuals at heightened risk for RA (e.g. first degree relatives of RA patients) may benefit from regular dental assessments and early treatment of periodontal disease, in addition to other lifestyle interventions (eg smoking cessation)

Research agenda:

It is essential to fully understand the pathophysiology of both RA and periodontitis to understand the inter-relationship between the two diseases and to find novel predictive markers of RA disease activity and progression. Some individual organisms such as *P. gingivalis* and *A. actinomycetemcomitans* are important but it is essential to consider the roles of imbalances of the composition and functions of the entire subgingival microbiome and, potentially, the blood microbiome. Further fundamental and translational research is required:

- To determine the influence of periodontal disease on the initiation and propagation of RA-autoimmunity. This will be best investigated in prospective cohorts of at-risk individuals including those with genetic risk (FDRs) and those with systemic autoimmunity
- To better understand the role of specific organisms such as *P. gingivalis* and *A. actinomycetemcomitans* as well as the entire subgingival microbiome in the development of localized and systemic RA-autoimmunity. To determine which organisms are associated with progression along the continuum from Pre-RA to established RA.

- To determine whether periodontal treatment should be considered as an adjunct to immunotherapy in patients with early RA.
- To conduct clinical trials to address whether treatment of periodontal disease and/or manipulation of the subgingival microbiome can delay or prevent RA in at-risk individuals.

Summary

Multiple studies have shown an epidemiological association between periodontitis and RA. Specific periodontal pathogens, *P. gingivalis* or *A. actinomycetemcomitans*, have been hypothesised to be of particular importance because they possess virulence determinants (PPAD and leukotoxin, respectively) that can contribute to the generation of citrullinated proteins and potentially trigger development of RA-related autoantibodies. However, periodontitis is a complex disease, mediated by consortia of co-operating bacteria and the host responses to them. Multiple mechanisms are likely to contribute to the association between periodontitis and RA and it is essential to consider the roles of imbalances of the composition and functions of the entire subgingival microbiome. Subgingival bacteria may contribute directly through enzymatic modification of proteins and subsequent autoantibody generation, or indirectly by dysregulation of neutrophils and enhancement of those neutrophil activities that contribute both to neo-epitope generation and host-mediated damage to periodontal tissues. It is possible that periodontal bacteria in the blood and hyper-active peripheral blood neutrophils may play a part in loss of immune tolerance and development of RA. Understanding the mechanisms underlying the inter-relationship between the two diseases and the influence of periodontitis and the periodontal microbiome on the initiation and propagation of RA-autoimmunity may help to identify novel predictive markers in individuals at risk of RA; it will inform clinical trials to determine if periodontal therapy should

be considered as an adjunct to immunotherapy in patients with early RA and whether treatment of periodontal disease and/or manipulation of the subgingival microbiome can delay or prevent RA in at-risk individuals.

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