Periodontitis: from microbial immune subversion to systemic inflammation

George Hajishengallis

Abstract | Periodontitis is a dysbiotic inflammatory disease with an adverse impact on systemic health. Recent studies have provided insights into the emergence and persistence of dysbiotic oral microbial communities that can mediate inflammatory pathology at local as well as distant sites. This Review discusses the mechanisms of microbial immune subversion that tip the balance from homeostasis to disease in oral or extra-oral sites.

Microbiota
A complex and diverse community of microorganisms that live within a given anatomical niche (such as an environmentally exposed surface of a multicellular eukaryotic organism).

Dysbiosis
A condition that is characterized by an imbalance in the relative abundance or influence of species within a microbial community that is associated with a disease; for instance, periodontitis or inflammatory bowel disease.

Homeostasis
A condition of equilibrium or stability in a system that is maintained by adjusting physiological processes to counteract external changes. An example of homeostasis is the balanced relationship between host tissues and the resident microbiota that prevents destructive inflammation or disease.

Department of Microbiology, Penn Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.
e-mail: geoh@upenn.edu
doi:10.1038/nri3785

Periodontitis is a chronic inflammatory disease that compromises the integrity of the tooth-supporting tissues, which include the gingiva, periodontal ligament and alveolar bone, and are collectively known as the periodontium[1] (BOX 1). Known since antiquity, periodontitis became prevalent after the domestication of plants and animals in Neolithic societies (~10,000 years ago) when the oral microbiota underwent a distinct compositional shift — with an increase in the frequency of *Porphyromonas gingivalis* and other periodontitis-associated species — compared with earlier hunter-gatherer societies[2]. In its severe form, which afflicts 8.5% of adults in the United States[3], periodontitis may not only cause tooth loss, but can also affect systemic health by increasing the patients' risk for atherosclerosis, adverse pregnancy outcomes, rheumatoid arthritis, aspiration pneumonia and cancer[4–9].

A triad of oral anaerobic bacteria that comprises *P. gingivalis*, *Treponema denticola* and *Tannerella forsythia* have traditionally been considered as causative agents of periodontitis, based on their virulence properties and strong association with diseased sites[10]. However, recent data from metagenomic, metatranscriptomic and mechanistic studies[11–16] are consistent with a new model of periodontal disease pathogenesis, which suggests that a more diverse periodontitis-associated microbiota is involved in the disease than previously thought. In this model, disease results not from individual pathogens but rather from polymicrobial synergy and dysbiosis, which perturb the ecologically balanced biofilm associated with periodontal tissue homeostasis[17–19] (FIG. 1). The dysbiosis of the periodontal microbiota is characterized by an imbalance in the relative abundance or influence of microbial species that have distinct roles which synergize to shape a pathogenic entity that can cause disease in the oral or extra-oral tissues of susceptible individuals[6,8,11,20]. In this new model of pathogenesis, the roles of individual bacteria and their interactions with the host need to be re-evaluated.

Central to the new model of the pathogenesis of periodontal disease, and constituting the main theme of this Review, is the active bacterial subversion of the host immune response in ways that enable the persistence of pathogens in the local inflammatory environment of periodontitis and the induction of pathology or complications at systemic sites. We discuss evidence regarding the mechanisms by which periodontal microorganisms disseminate from their oral habitat to distant sites — including atherosclerotic plaques, the lungs and the placenta — where they can disrupt immune surveillance and homeostasis to promote or accelerate pathogenic processes[9,21–29]. Moreover, *P. gingivalis* in particular is examined as a potential cause for the generation of autoantibodies in rheumatoid arthritis[5,30–33]. Understanding how oral pathogens misdirect the host immune response can provide novel mechanistic insights into the pathogenesis of periodontitis and associated systemic conditions, as well as reveal new therapeutic targets.

Microbial synergy and dysbiosis

The transition from periodontal health to disease is associated with a dramatic shift from a symbiotic microbial community — which is composed mostly of facultative bacterial genera such as *Actinomyces* and *Streptococci* — to a dysbiotic microbial community that is mainly composed of anaerobic genera from the phyla Firmicutes, Proteobacteria, Spirochaetes, Bacteroidetes and Synergistetes. The dysbiotic oral microbiota is enriched in virulence factors and has adapted to thrive in an inflammatory environment[11–14,34]. The predominant habitat of periodontitis-associated bacteria is the subgingival crevice, where the bacteria are found in

Box 1 | **Periodontitis and susceptibility factors**

Periodontitis has a complex aetiology that acts at multiple levels: at the microbial level, based on the presence of dysbiotic microbial communities with potential for destructive inflammation; at the host level, based on genetic factors that may predispose to or protect from disease; and at the level of systemic health status and environmental factors that modify the host response in either a protective direction or a destructive direction[151]. Accordingly, dysbiosis alone may not necessarily precipitate periodontitis, but it could initiate disease in the context of other risk factors, such as host genotype, stress, diet or behaviour (for example, smoking)[92,152–156]. For instance, there might be individuals who can tolerate dysbiosis by virtue of their intrinsic immuno-inflammatory status; hyporesponsive or lack-of-function polymorphisms in immune response genes could attenuate inflammation and prevent the development of overt disease[20]. Bacterial dysbiosis will only lead to disease in susceptible hosts as there are individuals who remain periodontally healthy despite massive tooth-associated biofilm formation, whereas others with less biofilm accumulation are extremely susceptible to periodontitis[154]. Although a genetic basis for periodontitis is supported by twin studies and familial aggregation of severe forms of the disease, there is debate concerning the role of specific genes, such as *IL1B* (which encodes interleukin-1β (IL-1β)), *IL6* (which encodes IL-6), *TNF* (which encodes tumour necrosis factor), *FCGR2A* (which encodes Fcγ receptor IIA), *C5* (which encodes complement component C5), *CD14* and *WNT5A*[153–155]. This uncertainty is probably attributable to the fact that chronic periodontitis is a polygenic disease, in which multiple genes contribute cumulatively to the overall disease risk (or protection) by influencing the host immune response and the composition and structure of the microbiota [155]. This notion stands in stark contrast to monogenic forms of the disease, such as aggressive periodontitis in young patients with leukocyte adhesion deficiency, in which a single gene (*ITGB2*; which encodes integrin β2) invariably precipitates periodontal disease[57].

distinct microenvironments — the tooth-associated biofilm, the gingival crevicular fluid and the epithelium lining the crevice[19] (FIG. 1a). The subgingival environment is rich in immune and inflammatory mediators, and provides unique challenges and opportunities for the bacteria[35–37]. Periodontal health requires a controlled immuno-inflammatory state that can maintain host–microorganism homeostasis in the periodontium[38]. However, in periodontitis, the host immune response is dysregulated — either because it is subverted by the microbial community or because of host immunoregulatory defects — and is therefore ineffective at restraining bacterial outgrowth and overt pathogenicity[19]. A poorly controlled host immune response can, in turn, generate a self-perpetuating pathogenic cycle, in which dysbiosis and inflammation reinforce each other by forming a positive feedback loop (FIG. 1b).

Most studies investigating microbial subversion of the periodontal host immune response have primarily used *P. gingivalis* as a model pathogen and therefore this pathogen is the focus of this Review. Decades of research have identified a plethora of documented or putative virulence factors of *P. gingivalis* that may contribute to its persistence in the subgingival region (reviewed in REFS 37,39). However, it is only now that we have begun to understand how this Gram-negative, asaccharolytic bacterium integrates its virulence attributes to enhance the pathogenicity of a polymicrobial community. Although *P. gingivalis* was thought to be capable of directly causing periodontitis in animal models[40], it is now established that *P. gingivalis*-induced periodontitis requires the presence of the commensal microbiota, as *P. gingivalis* is unable to cause periodontitis in germ-free mice despite colonizing this host[15]. In this context, *P. gingivalis* — although only present at a low frequency — is pathogenic owing to its ability to induce dysbiotic microbial communities and thereby act as a keystone pathogen[16,41]. Manipulation of the host immune response is fundamental to the capacity of *P. gingivalis* to instigate quantitative and qualitative alterations in the oral microbiota, which can thereby trigger inflammatory periodontal bone loss that is largely mediated by pathobionts[42–44]. Although non-pathogenic by themselves in the oral environment, certain commensals such as *Streptococcus gordonii* promote *P. gingivalis* colonization and, as such, are implicated as accessory pathogens[19,45].

It should be noted that the presence of *P. gingivalis* does not necessarily precipitate disease. Indeed, *P. gingivalis* is detectable, albeit at a decreased frequency, in periodontally healthy individuals[11,46]. This might be explained by the considerable strain diversity within the population of *P. gingivalis*. Moreover, key virulence factors of this pathogen (such as gingipains and lipid A phosphatases) are regulated by local environmental conditions, which may differ among individuals[41]. In a related context, there might be individuals who can resist the conversion of a symbiotic microbiota into a dysbiotic one by virtue of their intrinsic immune status, for example, owing to alterations in the signalling pathways that are required for immune subversion by *P. gingivalis* or other keystone-like pathogens.

The concept that *P. gingivalis* cooperates with other periodontal microorganisms is supported by findings in animal models of periodontitis in which combined inoculation of *P. gingivalis* with accessory pathogens or other keystone-like pathogens, such as *S. gordonii* or *T. forsythia*, respectively, leads to enhanced alveolar bone loss compared with inoculation of *P. gingivalis* alone[47–50]. This synergism may not be restricted to manipulation of the host response, but may also entail cooperative interspecies communication that further promotes bacterial fitness and hence dysbiosis. At least *in vitro*, communication among periodontal bacteria causes reciprocal transcriptomic and proteomic responses that regulate nutrient acquisition, metabolic processes and the production of virulence factors[51–53].

In summary, the emerging polymicrobial synergy and dysbiosis model suggests that the host immune response is initially subverted by keystone pathogens that are aided by accessory pathogens, and is subsequently overactivated by pathobionts, thereby linking the breakdown of homeostasis with destructive inflammation in susceptible individuals (FIG. 1b). Specific molecular mechanisms by which periodontal bacteria manipulate the host response to cause dysbiotic inflammation are discussed below.

Subversion of host immune responses

The dysbiotic periodontal community is faced with a survival conundrum: on the one hand, these bacteria need to evade immune-mediated killing; on the other hand, they require inflammation to procure nutrients from tissue breakdown (such as degraded collagen peptides and haem-containing compounds)[20]. Hence,

Subgingival crevice
Narrow space between the tooth surface and the free gingiva.

Gingival crevicular fluid
Serum exudate that originates in the gingival capillaries and flows into the gingival crevice carrying locally produced immune and inflammatory mediators such as complement, cytokines and antimicrobial peptides.

Keystone pathogen
A pathogen with a disproportionately large effect on its environment relative to its abundance; for example, low-abundance *P. gingivalis* remodels a commensal microbial community into a dysbiotic and disease-provoking microbiota.

Pathobionts
Commensal microorganisms with the potential to induce pathology under conditions of disrupted homeostasis.

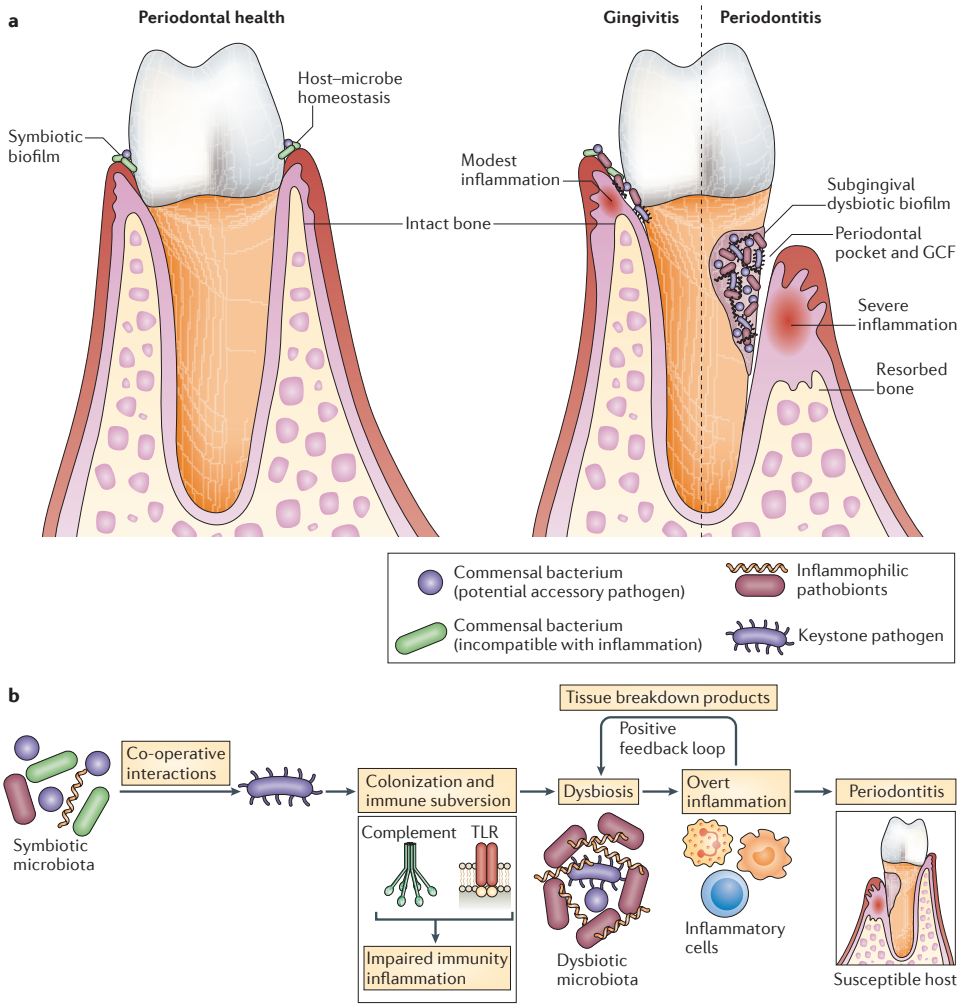


Figure 1 | **Polymicrobial synergy and dysbiosis in periodontitis.** **a** | The figure shows progression from a state of periodontal health, with a gingival crevice of ≤2 mm deep, to gingivitis, which is defined as periodontal inflammation without bone loss and is usually associated with a gingival crevice of ≤3 mm deep. Periodontitis develops when the formation of the periodontal pockets is ≥4 mm deep and the inflammatory response is associated with alveolar bone loss. Inflammation-induced collagenolytic enzymes can contribute to the loss of tissue attachment to the teeth, and the deepening and ulceration of the pockets that can become as deep as 10–12 mm and cover a surface area of 8–20 cm^2. These pockets act as a bacterial niche and can harbour 10^8—10^{10} bacteria that feed on inflammatory spoils such as collagen peptides and haem-containing compounds, which are carried with the gingival crevicular fluid (GCF) that bathes the pocket. **b** | Periodontitis is induced in susceptible hosts by a polymicrobial community, in which different members have distinct roles that converge synergistically to cause destructive inflammation. Keystone pathogens — the colonization of which is facilitated by accessory pathogens — initially subvert the host response leading to a dysbiotic microbiota, in which pathobionts overactivate the inflammatory response and cause periodontal tissue destruction, including resorption of the supporting alveolar bone. Inflammation and dysbiosis positively reinforce each other because inflammatory tissue breakdown products are used as nutrients by the dysbiotic microbiota. TLR, Toll-like receptor.

Accessory pathogens
Commensal bacteria that are not pathogenic by themselves in a given niche, but that can enhance the virulence of keystone pathogens by, for example, facilitating their colonization or providing metabolic support.

Gingipains
A family of trypsin-like cysteine proteinases that are secreted by *P. gingivalis* and contribute to its virulence, as well as to the pathogenesis of periodontitis. Members of this family include the high molecular mass arginine-specific gingipain A (HRgpA), the arginine-specific gingipain B (RgpB) and the lysine-specific gingipain (Kgp).

Inflammophilic
This term refers to bacteria that thrive on inflammation as they feed off inflammatory tissue breakdown products. The literal meaning is 'attracted to inflammation', which is derived from the combined meaning of inflammation and the Greek suffix *philic* denoting fondness.

Periodontal pockets
The pathologically deepened subgingival crevices in periodontitis, which are characteristic of the disease.

immunosuppression, although a common evasion strategy used by many pathogens[54], is not a viable option for inflammophilic bacteria[55]. Periodontal bacteria can manipulate their interactions with host immune responses — such as neutrophils and complement — to enhance bacterial fitness.

The role of neutrophils in periodontitis. Neutrophils are the most common leukocyte recruited to the subgingival crevice or periodontal pockets[35,36]. Individuals with congenital deficiencies in neutrophil numbers or recruitment develop severe periodontitis, suggesting that neutrophils are required for periodontal tissue homeostasis[17,56,57].

However, hyperactive, supernumerary or dysregulated neutrophils can cause collateral tissue damage through the release of inflammatory and toxic substances or tissue-degrading enzymes[35,58–60]. Indeed, ample clinical evidence shows that neutrophils mediate a significant amount of periodontal tissue destruction[61,62] and that their local numbers positively correlate with the severity of chronic periodontitis[63]. The chronic recruitment of excessive numbers of neutrophils to diseased periodontal pockets possibly arises from their inability to control the microbial challenge, despite being viable and capable of eliciting immune responses[20,58]. This suggests that the microorganisms can evade neutrophil-mediated killing while promoting inflammation, thereby contributing to dysbiosis. Indeed, as discussed below, periodontal bacteria can subvert complement function in ways that interfere with neutrophil-mediated killing in a persisting inflammatory environment[42].

Complement subversion. Complement is a cascade system involving a network of proteins and receptors that are crucially involved in immunity and inflammation[64]. Complement activation is initiated by distinct mechanisms, which converge at the third complement component (C3) and lead to the generation of effector molecules. These effector molecules mediate microbial opsonization and phagocytosis (for example, the opsonin C3b interacts with complement receptor 1); recruitment and activation of inflammatory cells (such as anaphylatoxin C5a, which interacts with the C5a receptor (C5aR)); and direct lysis of targeted microorganisms (mediated by the C5b–C9 membrane attack complex)[64]. *P. gingivalis*, *T. forsythia* and *Prevotella intermedia* can protect themselves and 'bystander' bacteria from human complement-mediated opsonophagocytosis and killing by blocking complement activation through the degradation of C3 or of key upstream components, such as the mannose-binding lectin[65–67]. Importantly, the bacterial proteases involved — namely, gingipains, karilysin and interpain A (which are expressed by *P. gingivalis, T. forsythia* and *P. intermedia*, respectively) — act synergistically to inhibit complement, which may lead to enhanced protection of complement-susceptible bystander species[65–67]. *P. intermedia* and *T. denticola* can also evade human complement-mediated killing by capturing complement factor H, which is a physiological inhibitor of complement[68,69].

Intriguingly, the arginine-specific gingipains of *P. gingivalis* can cleave C5 to generate high local concentrations of C5a independently of canonical complement activation[65,70]. Hence, in neutrophils, which recognize *P. gingivalis* through Toll-like receptor 2 (TLR2)[71], this pathogen can coactivate C5aR and TLR2, which in turn leads to signalling crosstalk[42]. In both human and mouse neutrophils, this C5aR–TLR2 crosstalk leads to ubiquitylation and proteasomal degradation of myeloid differentiation primary response protein 88 (MYD88), a TLR2 signalling adaptor, and thereby suppresses its antimicrobial effects[42] (FIG. 2). Moreover, the C5aR–TLR2 crosstalk activates an alternative pathway in which the TLR2 adaptor MYD88 adaptor-like protein (MAL; also known as TIRAP) induces phosphoinositide 3-kinase (PI3K) signalling, which in turn inhibits RHOA GTPase-dependent actin polymerization, and hence the phagocytosis of *P. gingivalis* and bystander bacteria[42]. The PI3K pathway also stimulates a robust inflammatory response[42] (FIG. 2). Importantly, the local inhibition of C5aR, TLR2 or PI3K in the periodontium of *P. gingivalis*-colonized mice leads to the elimination of *P. gingivalis*, reverses the increase in total microbiota numbers that are induced by *P. gingivalis* colonization and blocks periodontal inflammation[42]. In summary, *P. gingivalis* manipulates neutrophils through distinct mechanisms that together ensure the survival of the microbial community and the perpetuation of inflammation.

Interestingly, the karilysin of *T. forsythia* was recently shown to also cleave C5 to release C5a[66]. As *T. forsythia* activates TLR2 (REF. 72), it is important to determine whether this bacterium can instigate subversive crosstalk between C5aR and TLR2 similar to that of *P. gingivalis*, or whether the two microorganisms can synergize in that regard. Intriguingly, both gingipains and karilysin readily degrade the C5b component of C5, and thereby prevent the formation of the membrane attack complex[65,66].

Additional immune-subversive mechanisms. Major immune-subversive microorganisms probably use additional strategies to protect bystander bacteria and elevate the virulence of the entire microbial community, although most of these putative mechanisms have not been confirmed *in vivo*. For instance, the capacity of *P. gingivalis* to degrade and inactivate antimicrobial peptides might confer *in vivo* protection to bystander bacteria[36,67]. In a related context, *T. denticola* blocks the production of human β-defensins by gingival epithelial cells in response to *Fusobacterium nucleatum,* a microorganism that promotes the colonization of periodontitis-associated bacteria[73]. Mechanistically, *T. denticola* blocks the fusion of internalized *F. nucleatum* with lysosomes and suppresses the induction of intracellular reactive oxygen species, thereby inhibiting TLR responses that control human β-defensin expression[73]. Macrophages can engulf periodontal bacteria that invade the gingival connective tissue. However, *P. gingivalis* can induce cyclic AMP-dependent activation of protein kinase A, which inhibits the expression of inducible nitric oxide synthase, thereby suppressing nitric oxide-dependent intracellular killing in macrophages[74]. Moreover, *P. gingivalis* suppresses human and mouse macrophage endocytosis of *F. nucleatum*, an event that is required for NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome activation in response to this bacterium[75]. This mechanism may promote the fitness of the periodontal community as inflammasome activation induces pyroptosis — a pro-inflammatory mode of lytic cell death — protecting the host against infection[76]. *P. gingivalis* is also thought to manipulate adaptive immune responses by favouring the differentiation and recruitment of $CD4^+$ T helper 17 (T_H17) cells at the expense of the T_H1 lineage[77–79]. Although it has been argued that T_H17 cells contribute to destructive periodontal inflammation, whereas T_H1 cells are involved in protective cell-mediated immunity[80,81], more research is warranted to elucidate the roles of the various T cell subsets in the pathogenesis of periodontal disease.

Inflammasome
A cytosolic, multiprotein complex that responds to infection or tissue injury by activating pro-inflammatory caspases (mainly caspase 1), leading to the cleavage and release of pro-inflammatory cytokines (such as interleukin-1β (IL-1β) and IL-18) and under certain conditions, such as when myeloid cells are infected with pathogenic bacteria, leads to pyroptosis, a form of necrotic cell death.

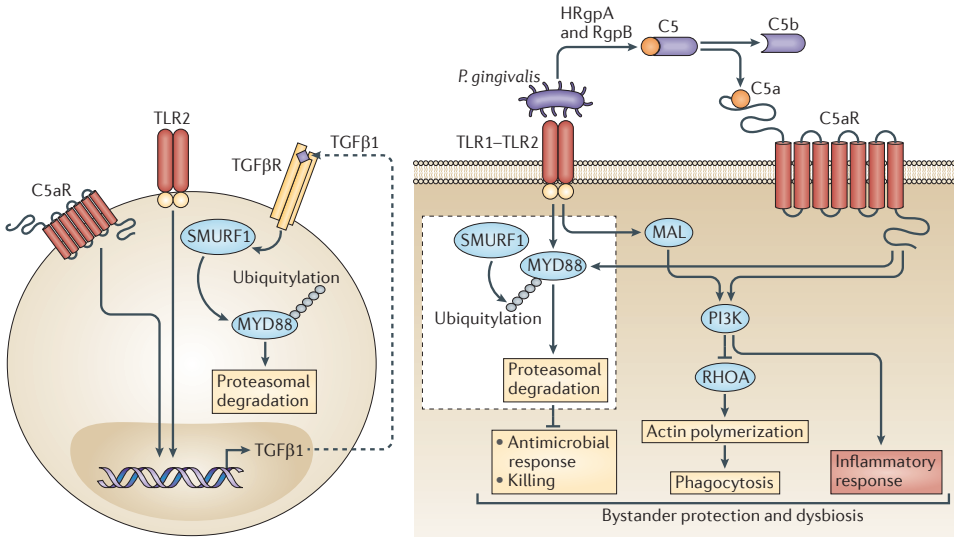


Figure 2 | ***Porphyromonas gingivalis* subversion of neutrophils leads to dysbiotic inflammation.** *Porphyromonas gingivalis* expresses ligands that activate the Toll-like receptor 1 (TLR1)–TLR2 complex and enzymes with C5 convertase-like activity (high molecular mass arginine-specific gingipain A (HRgpA) and arginine-specific gingipain B (RgpB)) that generate high local concentrations of C5a ligand. *P. gingivalis* can co-activate complement C5a receptor (C5aR) and TLR2 in neutrophils, and the resulting crosstalk leads to the ubiquitylation and proteasomal degradation of the TLR2 adaptor myeloid differentiation primary response protein 88 (MYD88), thereby inhibiting a host-protective antimicrobial response. This proteolytic event requires the C5aR–TLR2-dependent release of transforming growth factor β1 (TGFβ1), which mediates MYD88 ubiquitylation via the E3 ubiquitin-protein ligase SMURF1 (enlarged inset). Moreover, the C5aR–TLR2 crosstalk activates phosphoinositide 3-kinase (PI3K), which prevents phagocytosis through the inhibition of RHOA GTPase and actin polymerization, while stimulating the production of inflammatory cytokines. In contrast to MYD88, another TLR2 adaptor, MYD88-like adaptor protein (MAL), contributes to immune subversion by acting upstream of PI3K. These functionally integrated pathways, as manipulated by *P. gingivalis*, provide 'bystander' protection to otherwise susceptible bacterial species and promote polymicrobial dysbiotic inflammation *in vivo*. TGFβR, TGFβ receptor. Reprinted from *Cell Host Microbe* **15**, Maekawa, T. *et al. Porphyromonas gingivalis* manipulates complement and TLR signaling to uncouple bacterial clearance from inflammation and promote dysbiosis, 768–778, Copyright (2014), with permission from Elsevier.

When the periodontium is chronically exposed to a dysbiotic microbial community — which apparently evolved to evade the host immune response, while promoting its inflammatory aspects — it probably has an adverse impact on systemic health. Below, we examine the established and emerging mechanisms by which periodontal bacteria can subvert host signalling pathways to instigate chronic inflammation in extra-oral sites.

Periodontitis and cardiovascular disease

Numerous cross-sectional, case-control and cohort epidemiological studies suggest that periodontitis is associated with atherosclerotic cardiovascular disease, independently of confounding factors such as smoking and obesity[6,82,83]. Moreover, clinical interventional studies indicate that the treatment of periodontitis reduces systemic inflammation and has favourable effects on subclinical markers of atherosclerosis, including improved endothelial function (as determined by flow-mediated dilatation)[83–85]. Moreover, a recent study has shown that longitudinal improvement in periodontal health is related to decreased progression of carotid atherosclerosis in humans[86].

At least two biologically plausible mechanisms may account for a causative link between periodontitis and atherosclerosis[4,83] (FIG. 3). First, gingival ulceration in periodontal pockets (FIG. 1a) enables the translocation of bacteria into the systemic circulation, causing bacteraemia that is well documented in patients with periodontitis and that may provide an atherogenic stimulus[4,83]. This notion is supported by findings showing that recurrent experimental bacteraemia (using *P. gingivalis* as a model pathogen) promotes coronary and aortic atherogenesis in pigs with or without hypercholesterolaemia[87]. Additionally, in a subset of patients with severe periodontitis and an ulcerated gingival epithelium, locally produced pro-inflammatory cytokines — such as tumour necrosis factor (TNF), interleukin-1β (IL-1β) and IL-6 — can enter the systemic circulation and induce an acute-phase response in the liver (including elevated levels of C-reactive protein, fibrinogen and serum amyloid A) and thereby promote atherogenesis[4,88]. In support of this mechanism, patients with severe periodontitis have increased systemic inflammation — as indicated by elevated levels of cytokines and acute-phase markers, such as IL-6 and C-reactive protein,

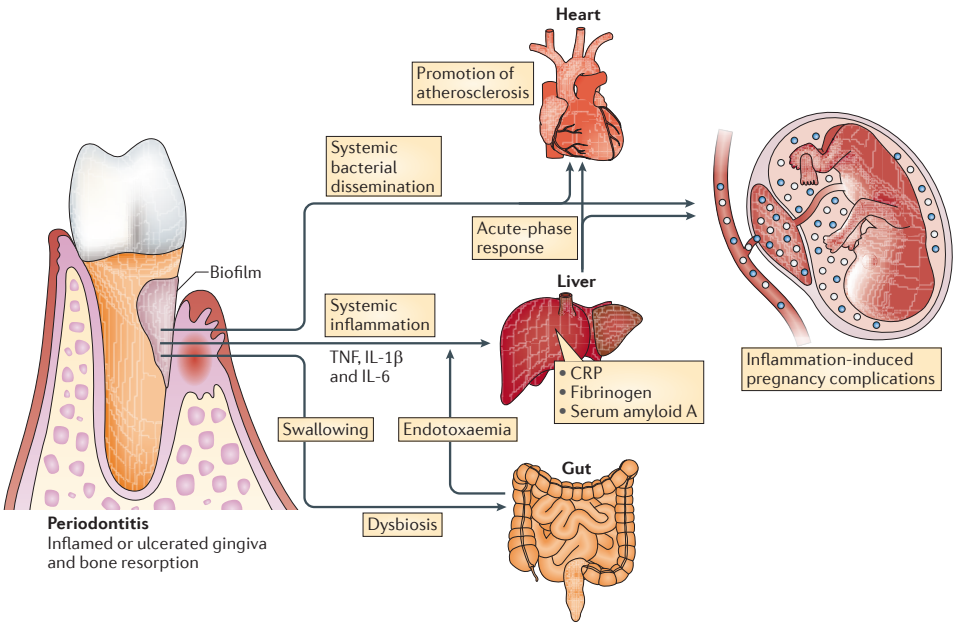


Figure 3 | **Biologically plausible mechanisms linking periodontitis to systemic inflammation and disease.** In periodontitis, locally produced pro-inflammatory cytokines can enter the systemic circulation and induce an acute-phase response in the liver — which is characterized by increased levels of C-reactive protein (CRP), fibrinogen and serum amyloid A — and in turn contribute to atherosclerosis or intra-uterine inflammation. Moreover, gingival ulceration in periodontal pockets enables the egress and systemic dissemination of periodontal bacteria. Certain bacteria, including *Porphyromonas gingivalis*, have been detected in circulating leukocytes and in atherosclerotic lesions, where they may act as pro-atherogenic stimuli. Other periodontal bacteria, such as *Fusobacterium nucleatum*, have been detected in the placenta where they can cause adverse pregnancy outcomes. Large quantities of oral bacteria are constantly swallowed via the saliva into the gut. In this context, an alternative or additional mechanism that links periodontitis to systemic inflammation was recently proposed; swallowed *P. gingivalis* may cause alterations to the gut microbiota, thereby leading to increased gut epithelial permeability and endotoxaemia, which causes systemic inflammation. Although independent, the depicted events are not mutually exclusive and could, in principle, occur simultaneously. The blue circles in the fetal–placental compartment indicate bacterial invasion and the white circles indicate the dissemination of inflammatory mediators. IL, interleukin; TNF, tumour necrosis factor.

respectively — compared with healthy controls, whereas treatment of periodontitis reduces systemic inflammation in patients with or without a history of cardiovascular disease[83,85]. An alternative mechanism was suggested by a recent study in mice, which showed that *P. gingivalis* can cause alterations to the gut microbiota, leading to indirect induction of systemic inflammation[89] (FIG. 3). Specifically, mice that were orally infected with *P. gingivalis* showed an increased proportion of Bacteroidetes and a decreased proportion of Firmicutes within their gut microbiota relative to sham-infected controls, which correlated with the decreased expression of tight-junction proteins in the ileum, and with the development of endotoxaemia and systemic inflammation. Although large quantities of oral bacteria are constantly swallowed via the saliva in humans and animals, *P. gingivalis* was not detected in the gut of infected mice; therefore, the mechanism by which it causes compositional changes to the gut microbiota remains uncertain. The mechanistic basis of the association between periodontitis and atherosclerosis is substantiated by studies in animal models that were based on oral infection with *P. gingivalis*, which has been detected in human atherosclerotic tissue, and by clinical or *in vitro* studies attesting to the atherogenic potential of periodontal bacteria[6,25–29,90–94].

Animal model-based evidence. Oral infection with *P. gingivalis* of rabbits fed a high-fat diet, or of atherosclerosis-prone (hyperlipidaemic) apolipoprotein E-deficient (*ApoE*$^{-/-}$) mice on a standard chow diet, causes not only local bone loss, but also systemic inflammation and atherosclerotic lesions[28,90,91]. The *ApoE*$^{-/-}$ mouse model of combined periodontitis and atherosclerosis has been extensively used and has provided insights into the role of TLR signalling in the disease process. Whereas TLR2 has an important role in mediating *P. gingivalis*-induced inflammatory atherosclerosis[95], the disease phenotype requires *P. gingivalis* to evade TLR4-mediated detection[29].

In this regard, *P. gingivalis* can enzymatically modify the lipid A moiety of its lipopolysaccharide to either evade or antagonize TLR4 activation in macrophages[17]. The shifting of lipid A activity from TLR4-evasive to

TLR4-antagonistic depends on endogenous lipid A phosphatase activity[96], which is regulated by the growth phase or by environmental factors including temperature and haemin availability[97,98]. Differential activities of lipid A 1′- and 4′-phosphatases are associated with the synthesis of distinct lipid A structures, comprising non-phosphorylated tetra-acylated lipid A (which is inert for TLR4 activation), monophosphorylated penta-acylated lipid A (which is a weak TLR4 agonist) and monophosphorylated tetra-acylated lipid A (a TLR4 antagonist). Genetic ablation of 4′-phosphatase activity (or bacterial growth at ≥39 °C) leads to the synthesis of TLR4-agonistic lipid A, whereas ablation of 1′-phosphatase activity — or growth in haemin-replete conditions, which are expected to be present in an inflammatory environment — leads to the synthesis of TLR4-antagonistic lipid A[96,99]. Remarkably, in addition to preventing TLR4 activation, the production of inert or antagonistic lipid A also increases the resistance of *P. gingivalis* to cationic antimicrobial peptides, owing to changes in the outer surface charge of the bacteria that affect the binding of cationic peptides[29,96,97,99]. Importantly, the TLR4-antagonistic lipid A of *P. gingivalis* inhibits TLR4 activation in response to other bacteria that express the TLR4-agonistic lipid A species[100,101].

In *ApoE*$^{-/-}$ mice orally infected with wild-type *P. gingivalis*, or isogenic phosphatase mutants with a 'locked' lipid A profile, the expression of inert or antagonistic lipid A was associated with elevated vascular inflammation, macrophage infiltration and the progression of atherosclerosis[29]. In macrophages infected with the same set of wild-type or mutant strains, the expression of lipid A structures that prevent or block TLR4 activation was associated with evasion of non-canonical inflammasome activation and increased bacterial survival, compared with the expression of TLR4-agonistic lipid A[29]. Taken together with an earlier study by the same group[95], these findings suggest that the capacity of *P. gingivalis* to prevent TLR4 signalling while activating TLR2 leads to atherogenic inflammation at sites distant from the initial infection. Using a similar *ApoE*$^{-/-}$ model, an independent study confirmed the causal link between periodontitis and atherosclerosis and, moreover, detected *P. gingivalis* in aortic tissues (including vascular endothelial cells) using fluorescent *in situ* hybridization[28].

Immune subversion and atherogenic potential. Although periodontal bacteria have been identified in atheromas[25–27,92–94], the mode of their relocation is uncertain and could involve mechanisms alternative or additional to the bacteraemic route. In principle, bacteria might exploit recirculating leukocytes — such as macrophages and/or dendritic cells (DCs) — as 'Trojan horses' for dissemination to systemic tissues. In this regard, the ability of *P. gingivalis* to survive intracellularly in macrophages[29,102] and DCs[27] is intriguing. To persist intracellularly in macrophages, *P. gingivalis* needs to enter the macrophage via complement receptor 3 in cholesterol-rich lipid rafts[102,103], which is consistent with observations that pathogens which invade through lipid rafts are not readily directed to late endosomes and lysosomes, where they would be killed[104]. To survive within DCs, *P. gingivalis* needs to enter via the C-type lectin DC-specific ICAM3-grabbing non-integrin (DC-SIGN; also known as CD209)[105,106] (FIG. 4). Both processes are mediated by the fimbrial proteins of *P. gingivalis*; FimA fimbriae directly interact with complement receptor 3 on macrophages, whereas Mfa1 fimbriae interact with DC-SIGN on DCs[102,106].

The exploitation of DCs as transport vehicles for *P. gingivalis* is supported by a recent clinical study that identified *P. gingivalis* (using 16S ribosomal DNA sequencing) within blood myeloid DCs from patients with chronic periodontitis[27]. The rate and frequency of DC carriage of *P. gingivalis* was increased after bacteraemia elicited by debridement, which is a routine clinical procedure that involves the removal of the dental plaque biofilm and calculus from the teeth and gingiva. Moreover, immunofluorescence analysis revealed colocalization of *P. gingivalis* with myeloid DCs that were infiltrating either the oral mucosa or atherosclerotic plaques of patients with chronic periodontitis[27].

A mechanistic understanding of these clinical observations was provided by *in vitro* studies showing that *P. gingivalis* subverts human DC function[27,105,106]. Specifically, following binding to DC-SIGN, *P. gingivalis* not only enters and survives within myeloid DCs, but also promotes an atherogenic phenotype in the cells, as indicated by the upregulation of matrix metalloproteinase 9 and complement C1q proteins, which are indicators of the risk of plaque rupture[27]. Furthermore, *P. gingivalis* selectively upregulates the expression of CXC-chemokine receptor 4 (CXCR4), but not that of CC-chemokine receptor 7 (CCR7), and thereby disrupts DC migration towards CC-chemokine ligand 19 (CCL19) and promotes migration towards CXC-chemokine ligand 12 (CXCL12)[107]. Consistent with this, peripheral blood myeloid DCs from patients with chronic periodontitis are characterized by high expression of CXCR4 and low expression of CCR7 compared with DCs from healthy individuals[107]. CCR7 mediates homing to secondary lymphoid organs, whereas CXCR4 mediates homing to sites of neovascularization, such as atherosclerotic plaques[108,109]; therefore, the authors suggested that *P. gingivalis* hijacks and directs DC migration to inflammatory vascular sites, where the pathogen can exacerbate inflammatory pathology[107].

An alternative transport vehicle for *P. gingivalis* in the blood might be erythrocytes, which bind to C3b-opsonized *P. gingivalis* in a complement receptor-1-dependent manner[110] (FIG. 4). Although leukocytes fail to bind and internalize erythrocyte-attached *P. gingivalis*, this may not necessarily be an evasion strategy, as erythrocyte-associated bacteria can eventually be cleared by liver macrophages (also known as Kupffer cells). However, because the interaction of *P. gingivalis* with erythrocytes gradually declines over time — which is attributed to bacterial degradation of bound C3b — resulting in the release of viable bacteria *in vitro*, the authors speculated that erythrocyte-carried *P. gingivalis* can potentially infect endothelial cells at extra-oral sites and thereby contribute to vascular inflammation[110] (FIG. 4). This intriguing hypothesis remains to be tested in animal models.

Non-canonical inflammasome
A caspase-11-dependent pathway of inflammasome activation that is crucial for the control of infections caused by Gram-negative bacteria and can induce cell death (pyroptosis) independently of caspase 1.

Atheromas
Accumulated fatty deposits in the inner lining (intima) of an artery that lead to the restriction of blood flow and an increased risk of thrombosis.

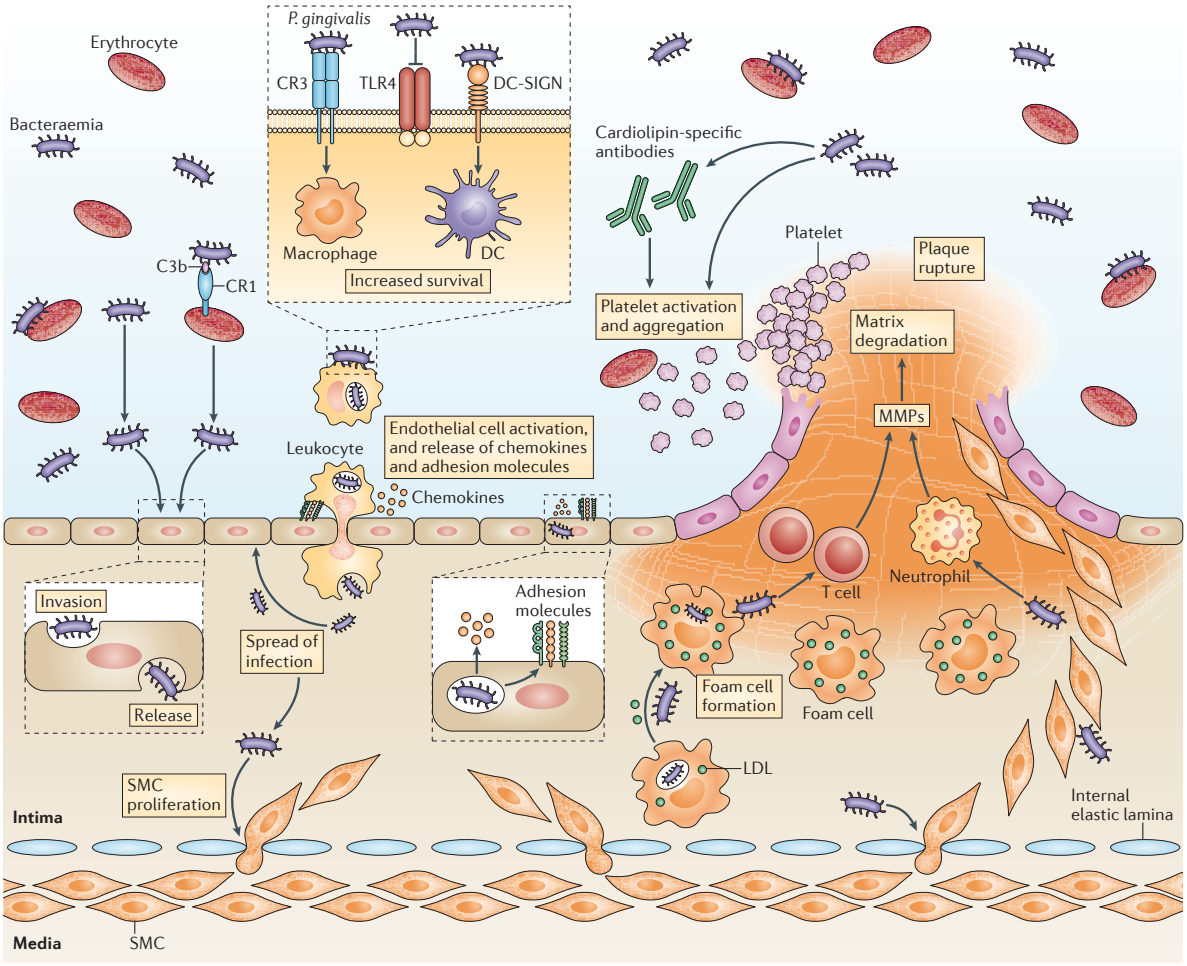


Figure 4 | **Microbial immune subversion in atherogenesis.** In addition to a bacteraemic route, periodontal bacteria may hijack leukocytes or erythrocytes — to which they attach via a C3b–complement receptor 1 (CR1) interaction — to disseminate from the oral mucosa to aortic tissues. Bacteria not only invade but also activate endothelial cells by upregulating cell adhesion molecules and chemokines, which can promote the transmigration of leukocytes that may harbour viable intracellular bacteria. The bacteria can spread to deeper tissues where they can induce the proliferation of smooth muscle cells (SMCs) in the intima. The uptake of low-density lipoproteins (LDLs) by transmigrated macrophages is enhanced in the presence of bacteria, leading to accelerated foam cell formation and atherogenesis. At later stages, atherosclerotic plaque rupture can be facilitated by the bacteria-induced production of matrix metalloproteinases (MMPs) by lymphocytes or myeloid cells. Bacteria-induced platelet aggregation — occurring either directly or through the induction of prothrombotic autoantibodies — may contribute to thrombotic vessel occlusion. Most of the studies supporting this model used *Porphyromonas gingivalis* as a model pathogen; the survival of this pathogen within leukocytes depends partly on Toll-like receptor 4 (TLR4) evasion, as well as on its capacity to exploit CR3 in macrophages or DC-specific ICAM3-grabbing non-integrin (DC-SIGN) in dendritic cells (DCs) for safe intracellular entry.

P. gingivalis can indeed invade human aortic endothelial cells by means of its FimA fimbriae and subsequently suppress the levels of key intracellular molecules that are involved in cell death and host defence (such as NLRP3 and receptor-interacting serine/threonine-protein kinase 1), which leads to a permissive intracellular environment[6,94,111,112]. FimA fimbriae also induce the TLR2-dependent expression of endothelial adhesion molecules (such as intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1) and E-selectin) and chemokines (such as MCP1 (also known as CCL2) and CXCL8 (also known as IL-8)) that are involved in leukocyte recruitment, as well as inside-out TLR2 signalling, which activates leukocyte integrins that mediate transendothelial migration[6,113,114]. However, additional TLR2 ligands of *P. gingivalis*, including serine lipids and lipoproteins[115,116], probably also participate in these pro-atherogenic activities.

Other studies using *P. gingivalis* as a pro-atherogenic model bacterium have shown that it can accelerate atherothrombosis via the recruitment and activation of neutrophils[117], and can induce platelet aggregation — an activity facilitated by the interactions of *P. gingivalis* haemagglutinins with the platelet glycoprotein IIb–IIIa (also known as αIIbβ3 integrin) on platelets via a fibrinogen bridge[118]. *P. gingivalis* may also contribute to cardiovascular disease pathology via molecular mimicry. Specifically, when compared with healthy individuals, a substantial subset of patients with periodontitis have elevated concentrations of cardiolipin-specific antibodies in the gingival crevicular fluid and serum; these are prothrombotic autoantibodies associated with atherosclerosis and also with adverse pregnancy outcomes (see discussion below). Such autoantibodies can be induced in response to bacterial epitopes — such as those found in *P. gingivalis* arginine-specific gingipains and *T. denticola* phosphoglycerate kinase — that bear homology to the TLRVYK peptide of the phospholipid-binding serum protein β2-glycoprotein 1 (REFS 119,120). Furthermore, *P. gingivalis* enhances low-density lipoprotein uptake and foam cell formation by upregulating the expression of CD36 (a scavenger receptor mediating lipid uptake; also known as platelet glycoprotein 4) and downregulating the expression of ABC transporter A family member 1 (which mediates the efflux of cholesterol from macrophages)[121,122]. In a similar context, the pathogen induces the proliferation of vascular smooth muscle cells, which leads to neointima formation, and stimulates the production of matrix metalloproteinases that contribute to plaque rupture and thrombotic vessel occlusion[6] (FIG. 4).

In summary, periodontal pathogens evade the immune system to induce atherogenic inflammation, although their impact beyond the oral cavity is not restricted to the cardiovascular system but includes additional tissues (see below).

Periodontitis and adverse pregnancy outcomes

Epidemiological and clinical studies suggest that maternal periodontitis may be associated with an increased risk of adverse pregnancy outcomes such as low birth weight, pre-term birth, miscarriage and/or stillbirth[9,21,123]. Similar to atherosclerosis, two major plausible biological mechanisms have been proposed: first, periodontal pathogens that disseminate systemically may cross the placenta into the fetal circulation and amniotic fluid; and second, inflammatory mediators that are produced locally in the periodontium could enter the systemic circulation and stimulate an acute-phase response, and thereby adversely affect the placenta and fetus (FIG. 3). The notion that periodontal bacteria can cause pregnancy complications is supported by mechanistic studies in animal models.

F. nucleatum — a potential accessory pathogen that facilitates the colonization of periodontitis-associated bacteria — becomes an overt pathogen if it translocates to extra-oral sites[8]. Indeed, the bacterium can be isolated from abscesses in several internal organs and it is implicated in adverse pregnancy outcomes and in colorectal cancer (BOX 2). Clinical studies have linked *F. nucleatum* to several pregnancy complications, including premature birth, stillbirth and neonatal sepsis[8]. Moreover, the identification of *F. nucleatum* clones in the subgingival biofilm of a mother with pregnancy-associated gingivitis and her stillborn infant suggests that the bacterium can disseminate from the mother's gingiva to her uterus[21]. Experiments in pregnant mice have provided insights into how *F. nucleatum* can cause intra-uterine infection and inflammation. Specifically, intravenously administered *F. nucleatum* (to mimic bacteraemia) uses its E-cadherin-binding FadA adhesin to cross the endothelium and colonize the fetal–placental compartment, where it induces TLR4-dependent necro-inflammatory responses[124]. In contrast to *F. nucleatum*, *Escherichia coli* fails to induce fetal loss in the same model[125]. Whereas *F. nucleatum* can colonize the placenta of TLR4-deficient mice, these mice display substantially decreased fetal death rates compared with wild-type mice, which indicates that fetal loss is caused by inflammation rather than by bacterial colonization per se[124].

Certain other periodontal bacteria, including *P. gingivalis,* were also shown to colonize the placenta and fetal tissues of mice or rats, thereby causing inflammation and pregnancy complications (reviewed in REF. 9). *P. gingivalis* may also induce adverse pregnancy outcomes through alternative mechanisms. Cardiolipin-specific antibodies are associated with certain disorders, including adverse pregnancy outcomes[119], and a subset of patients with periodontitis have increased concentrations of such autoantibodies[126], which could be induced in response to cross-reactive bacterial epitopes such as the arginine-specific gingipains of *P. gingivalis*[119]. The possible connection between *P. gingivalis*, cardiolipin-specific antibodies and pregnancy complications was strengthened recently. A study showed that antibodies raised against *P. gingivalis* — but not against an isogenic gingipain-deficient mutant — cause fetal loss when passively administered to pregnant female mice[127]. Importantly, this effect was substantially inhibited when cardiolipin-specific antibodies were removed from the antibody preparations[127].

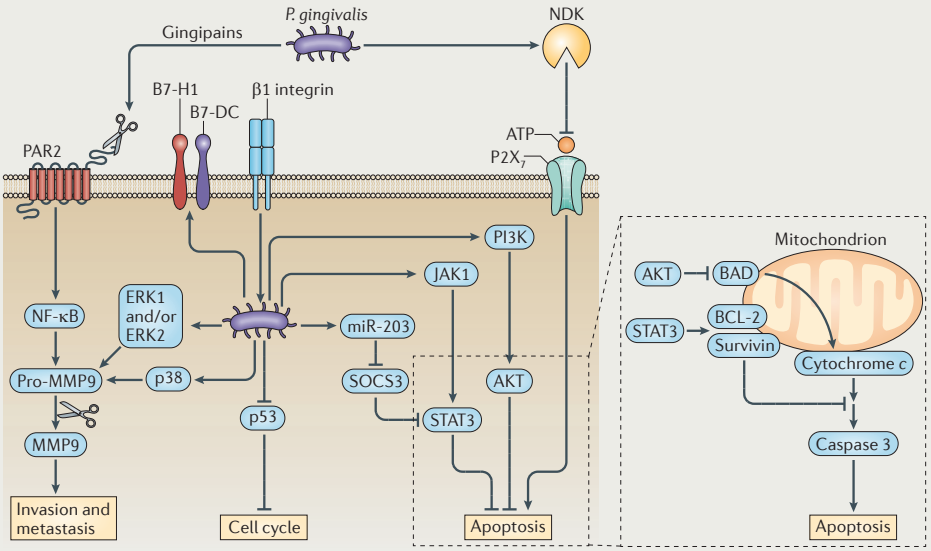
In summary, pregnancy complications can be caused by periodontal pathogen-induced inflammatory responses, or by autoantibodies, which have also been implicated in the association of periodontitis with rheumatoid arthritis.

P. gingivalis and rheumatoid arthritis

Several studies indicate an epidemiological association between periodontitis and rheumatoid arthritis, even after adjusting for common risk factors such as smoking[128–130]. In a distinct clinical phenotype of rheumatoid arthritis, anti-citrullinated protein antibodies (ACPAs) act as diagnostic markers as they are detected in the serum before the onset of the disease and their serum levels correlate strongly with disease severity[131]. A recent study showed that patients with rheumatoid arthritis — particularly those with ACPA-positive rheumatoid arthritis — have a higher incidence of periodontitis than control patients with osteoarthritis. Furthermore, in these patients, the detection of *P. gingivalis* in subgingival biofilm samples was associated with increased levels of ACPAs, irrespective of smoking status[32]. The molecular underpinnings of these associations are discussed below.

Box 2 | **Oral bacteria and cancer**

Accumulating evidence indicates that a temporal and spatial association exists between oral bacteria and cancer[7,157]. *Fusobacterium nucleatum* is associated with colorectal cancer[158], which has been attributed to its ability to stimulate the growth of colorectal cancer cells[159]. This activity depends on the interactions between E-cadherin and the *F. nucleatum* adhesin FadA; the expression of FadA is elevated in the cancerous colon tissue and correlates with the expression of oncogenic and inflammatory genes[159]. *Porphyromonas gingivalis* is associated with oral squamous cell carcinoma (OSCC)[160,161], orodigestive cancer (independently of periodontitis)[162] and pancreatic cancer[163]. Certain immune-subversive mechanisms of *P. gingivalis* are consistent with a role in cancer development (see the figure). In OSCC cells, *P. gingivalis* induces the expression of the pro-form of matrix metalloproteinase 9 (pro-MMP9) by triggering proteinase-activated receptor 2 (PAR2)-mediated nuclear factor-κB (NF-κB) activation (through an extracellular mechanism involving gingipain secretion) or by activating extracellular signal-regulated kinase 1 (ERK1)–ERK2 and p38 mitogen-activated protein kinase (MAPK) pathways, through an intracellular mechanism requiring β1-integrin-dependent invasion[164]. In addition, the gingipains cleave the secreted pro-enzyme into mature MMP9 (also known as gelatinase), which promotes carcinoma cell migration[164]. *P. gingivalis* invasion of epithelial cells suppresses apoptosis and stimulates cell proliferation by inhibiting the tumour suppressor p53 (REF. 165). The ability of *P. gingivalis* to activate the Janus kinase 1 (JAK1) and signal transducer and activator of transcription 3 (STAT3) pathway, as well as the phosphoinositide 3-kinase (PI3K) –AKT signalling pathway, causes inhibition of the intrinsic mitochondrial apoptosis pathways[149,166]. Specifically, STAT3, which upregulates the anti-apoptotic molecules survivin and B cell lymphoma 2 (BCL-2), and AKT, which inhibits the pro-apoptotic molecule BCL-2-associated agonist of cell death (BAD), lead to caspase 3 inhibition (see the inset)[148,149]. Moreover, extracellular release of nucleoside diphosphate kinase (NDK) by *P. gingivalis* cleaves ATP and prevents the induction of apoptosis via the P2X purinergic receptor 7 ($P2X_7$)[167]. Although suppressor of cytokine signalling 3 (SOCS3) can induce apoptosis by targeting STAT3 (REF. 168), *P. gingivalis* upregulates the microRNA miR-203 which directly inhibits SOCS3 (REF. 169). *P. gingivalis* can additionally induce B7 family ligands, such as B7-H1 (also known as PDL1 and CD274) and B7-DC (also known as PDCD1LG2), that interact with programmed cell death protein 1 on T cells[170] (not shown) potentially leading to the suppression of T cell function[171].



Peptidyl-arginine deiminase. *P. gingivalis* is unique among other periodontal bacteria, and possibly among all bacterial species, with regard to its expression of peptidyl-arginine deiminase (PPAD), which is an enzyme that converts protein arginine residues into citrulline[5,33]. Protein citrullination has been implicated in several physiological processes[132]; however, because citrullination can dramatically alter protein structure and function, dysregulated citrullination of host proteins by microbial enzymes could interfere with normal host cell signalling and immune or other homeostatic functions. For instance, *P. gingivalis* PPAD citrullinates the carboxy-terminal arginine of epidermal growth factor (EGF), thereby inhibiting its biological activity, as shown by impaired EGF-induced fibroblast proliferation and migration[133]. Interestingly, citrullination of two internal arginine residues of EGF by human peptidyl-arginine deiminase (PAD) enzymes does not abrogate EGF function[133]. As EGF has an essential role in wound healing and tissue regeneration, its inactivation by *P. gingivalis* may interfere with the healing of periodontal tissue and thus delay the resolution of inflammation.

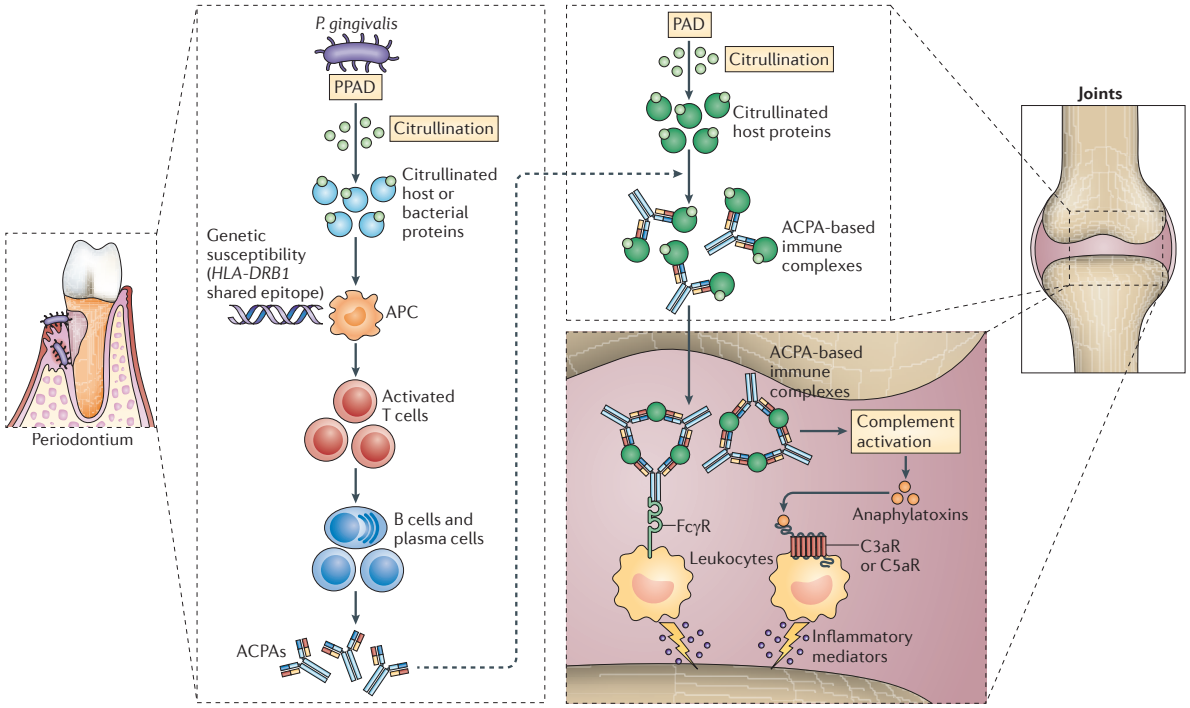


Figure 5 | ***Porphyromonas gingivalis*-mediated citrullination and induction of anti-citrullinated protein antibodies in rheumatoid arthritis.** *Porphyromonas gingivalis* peptidyl-arginine deiminase (PPAD) citrullinates host-derived or bacterial proteins in the inflammatory environment of periodontitis. In susceptible individuals who are carriers of the *HLA-DRB1* shared epitope alleles, distinct citrullinated peptides are presented by antigen-presenting cells (APCs) in the context of *HLA-DRB1* shared epitope alleles to activate T cells, which in turn stimulate B cell production of anti-citrullinated protein antibodies (ACPAs). The induction of autoantibodies may be explained by mechanisms that involve neoepitope formation or molecular mimicry. Citrullination of host proteins — such as α-enolase, fibrinogen and collagen type II — by human peptidyl-arginine deiminase (PAD) enzymes can occur in injured or inflamed joints. ACPAs bind to citrullinated proteins (indicated by the dashed arrow) and form immune complexes that can mediate local synovial inflammation by activating complement or Fc receptors for IgG (FcγRs). C3aR, C3a receptor; C5aR, C5a receptor.

Link between P. gingivalis and rheumatoid arthritis. The unique ability of *P. gingivalis* to citrullinate proteins has attracted considerable interest in the field of rheumatoid arthritis given the importance of ACPAs in disease pathogenesis[5,131]. In this context, *P. gingivalis* was shown to citrullinate human fibrinogen and α-enolase, which, in their citrullinated forms, are two major rheumatoid arthritis autoantigens[31]. This activity requires concerted action between PPAD and arginine-specific gingipains, which colocalize with PPAD in the outer membrane of *P. gingivalis*[31]. Specifically, the cleavage of fibrinogen or α-enolase by gingipains exposes C-terminal arginine residues that are subsequently citrullinated by PPAD[31]. In principle, the unique mode of proteolytic processing and post-translational modification of host antigens by *P. gingivalis* could generate neoepitopes to which immune tolerance does not exist, which would lead to the generation of autoantibodies (FIG. 5). It should be noted that the breakdown of immune tolerance to citrullinated proteins requires susceptible individuals, such as carriers of *HLA-DRB1* shared epitope alleles that bind selectively to citrullinated sequences and may influence antigen presentation in ways that lead to ACPA production[134]. Intriguingly, rheumatoid arthritis-specific autoantibodies to citrullinated α-enolase peptide 1 (the immunodominant B cell epitope of human α-enolase) were shown to cross-react with citrullinated enolase from *P. gingivalis*, suggesting that molecular mimicry can contribute to autoantibody generation[135].

A 'two-hit' model of rheumatoid arthritis pathogenesis was proposed involving the initial breakdown of tolerance to citrullinated peptides generated by *P. gingivalis* in inflamed gingiva, followed by epitope spreading to other host-citrullinated proteins in the inflamed joint[5,31] (FIG. 5). In this regard, citrullinated proteins have been detected in the gingiva of patients with periodontitis[5]. Moreover, the notion that immunity to citrullinated proteins is initially triggered in inflamed mucosal surfaces that are distant from the joints is consistent with the presence of ACPAs before signs of inflammation in the joints[136]. Mechanisms of epitope spreading or molecular mimicry could lead to cross-reactivity with citrullinated joint proteins and the subsequent formation of immune complexes that could exacerbate or perpetuate the inflammatory process in

rheumatoid arthritis through several mechanisms, including activation of complement or Fc receptors for IgG (Fcγ receptors)[5,31] (FIG. 5). Epitope spreading precedes the development of rheumatoid arthritis and leads to the maintenance and progression of inflammation as it sustains the generation of high-affinity ACPAs to citrullinated host proteins[5,137].

The two-hit model is consistent with the results of two recent mechanistic studies by independent groups. Specifically, infection of mice with wild-type *P. gingivalis* exacerbates collagen- or collagen-antibody-induced arthritis, as shown by the accelerated progression and enhanced severity of bone and cartilage destruction[30,138]. The ability of wild-type *P. gingivalis* strains to aggravate arthritis is strictly dependent on PPAD expression, as isogenic mutants lacking this enzyme fail to influence disease outcome[30,138]. Furthermore, the infection of mice with wild-type, but not with PPAD-deficient, *P. gingivalis* was associated with the detection of citrullinated proteins at the site of infection, and with the production of ACPAs[30,138]. These findings suggest that, by virtue of its PPAD, *P. gingivalis* may constitute a mechanistic link between periodontitis and rheumatoid arthritis.

Periodontitis and respiratory diseases

Aspiration pneumonia. The tooth-associated bacterial biofilm is thought to be a reservoir for respiratory infections, and oral anaerobic bacteria are common isolates from aspiration pneumonia and lung abscesses[22,139,140]. Oropharyngeal aspiration of bacteria is a major cause of pneumonia in older people and immunocompromised individuals[139,140], and periodontitis is epidemiologically implicated as a mortality risk factor for aspiration pneumonia, at least in the elderly[141]. As patients probably aspirate fragments of the biofilm, which is composed of mixed bacterial species, the synergistic polymicrobial interactions that are seen in periodontitis might also occur in the lung tissue. Despite limited research in this area, mixed infection with *P. gingivalis* and *T. denticola* in a mouse model of aspiration pneumonia has been shown to cause considerably increased inflammatory responses, impaired bacterial clearance and more severe lung pathology compared with single infection with either bacterium[142]. Importantly, the control of the oral microbial burden substantially decreases the incidence of aspiration pneumonia in frail elderly people[143,144], which suggests a direct association between oral bacteria and lung pathology in susceptible individuals. These results warrant more basic studies to investigate the mechanisms involved.

Chronic obstructive pulmonary disease. Periodontitis is also associated with chronic obstructive pulmonary disease (COPD)[23,145]. Polymicrobial infections, including infections with the opportunistic pathogen *Pseudomonas aeruginosa*, are associated with exacerbations of COPD and increase its morbidity and mortality[23,146]. *P. gingivalis* is readily detectable with *P. aeruginosa* in tracheal aspirates of patients with acute COPD exacerbations[24], and can enhance the pathogenicity of *P. aeruginosa* in the context of lower airway infection[147,148]. Indeed, *P. gingivalis* promotes the ability of *P. aeruginosa* to invade respiratory epithelial cells and modulates its apoptosis-inducing capacity[147,148]. Specifically, compared with the invasion of *P. aeruginosa* alone, co-invasion of the respiratory epithelial cells with both bacteria leads to diminished apoptosis as a result of the enhanced signalling of signal transducer and activator of transcription 3 (STAT3), which upregulates the expression of the anti-apoptotic molecules survivin and B cell lymphoma 2 (BCL-2), and in turn leads to the inhibition of caspase 3 (REF. 148). Moreover, co-invasion is followed by the downregulation of the pro-apoptotic molecule BCL-2-associated agonist of cell death (BAD)[148], which is possibly mediated by PI3K–AKT signalling[149] (see the figure in BOX 2). However, the inhibition of apoptosis is transient as the signalling pathways that are involved start to decline 8 hours post-invasion of both bacteria, leading to dramatically increased caspase 3 activity by 12 hours post-invasion[148]. Rapid apoptosis of infected epithelial cells is thought to contribute to the effective clearance of *P. aeruginosa*[150]. This suggests that the inhibition of epithelial cell apoptosis for a substantial amount of time following *P. aeruginosa* and *P. gingivalis* co-invasion may provide the bacteria with a safe intracellular niche and the opportunity to proliferate and establish infection.

Conclusions and perspective

Dysbiotic microbial communities in the periodontium resist immune elimination and create permissive conditions for growth in a nutritionally favourable inflammatory environment[11,13,17–19,41,42] (FIGS 1,2). The immune-subversive and pro-inflammatory strategies that promote the fitness of periodontal bacteria not only cause collateral damage to the periodontium, but also have repercussions that link periodontitis to systemic afflictions (FIGS 3–5). The virulence of individual periodontal pathogens is maximized in the context of a polymicrobial infection[17,19,47–51,53], and its impact on the host depends on genetic predisposition and environmental modifiers[92,151–155] (BOX 1). Hence, to better understand the mechanisms of the pathogenesis of periodontitis and associated systemic conditions, data from epidemiological and animal model studies need to be integrated with those from metatranscriptomic and metaproteomic approaches, as well as with data from whole-genome transcriptomic and proteomic analyses of tissue samples from healthy individuals and from patients with different stages of disease. This integration can offer insights into the dynamic nature of host–microorganism interactions in disease development and, moreover, can facilitate the formulation of novel hypotheses for further studies. More importantly, key findings from basic research need to be translated into clinical applications and inform the development of therapies that counteract the immune-subversive mechanisms of periodontal bacteria, thereby contributing to the treatment of periodontitis and associated systemic inflammatory disorders. Such host-modulation strategies are more likely to succeed than direct antimicrobial approaches, particularly when targeting keystone pathogens, which can act at low abundance and will probably not be completely eradicated, partly because they can hide within permissive host cells.

1. Pihlstrom, B. L., Michalowicz, B. S. & Johnson, N. W. Periodontal diseases. *Lancet* **366**, 1809–1820 (2005).
2. Adler, C. J. *et al.* Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nature Genet.* **45**, 450–455 (2013).
3. Eke, P. I., Dye, B. A., Wei, L., Thornton-Evans, G. O. & Genco, R. J. Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J. Dent. Res.* **91**, 914–920 (2012).
4. Genco, R. J. & Van Dyke, T. E. Prevention: reducing the risk of CVD in patients with periodontitis. *Nature Rev. Cardiol.* **7**, 479–480 (2010).
5. Lundberg, K., Wegner, N., Yucel-Lindberg, T. & Venables, P. J. Periodontitis in RA — the citrullinated enolase connection. *Nature Rev. Rheumatol.* **6**, 727–730 (2010).
6. Kebschull, M., Demmer, R. T. & Papapanou, P. N. "Gum bug leave my heart alone": epidemiologic and mechanistic evidence linking periodontal infections and atherosclerosis. *J. Dent. Res.* **89**, 879–902 (2010).
7. Whitmore, S. E. & Lamont, R. J. Oral bacteria and cancer. *PLoS Pathog.* **10**, e1003933 (2014).
8. Han, Y. W. & Wang, X. Mobile microbiome: oral bacteria in extra-oral infections and inflammation. *J. Dent. Res.* **92**, 485–491 (2013).
9. Madianos, P. N., Bobetsis, Y. A. & Offenbacher, S. Adverse pregnancy outcomes (APOs) and periodontal disease: pathogenic mechanisms. *J. Clin. Periodontol.* **40**, S170–S180 (2013).
10. Socransky, S. S. & Haffajee, A. D. Periodontal microbial ecology. *Periodontol. 2000* **38**, 135–187 (2005).
11. Abusleme, L. *et al.* The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. *ISME J.* **7**, 1016–1025 (2013).
This metagenomic study characterizes ecological events in the subgingival microbial communities that underpin the development of periodontitis, and elucidates the relationship between inflammation and the disease-associated microbiome.
12. Griffen, A. L. *et al.* Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. *ISME J.* **6**, 1176–1185 (2012).
13. Jorth, P. *et al.* Metatranscriptomics of the human oral microbiome during health and disease. *MBio* **5**, e01012–e01014 (2014).
14. Dewhirst, F. E. *et al.* The human oral microbiome. *J. Bacteriol.* **192**, 5002–5017 (2010).
This study led to the Human Oral Microbiome Database and was the first curated description of a human body-site-specific microbiome.
15. Hajishengallis, G. *et al.* Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host Microbe* **10**, 497–506 (2011).
This paper provides *in vivo* evidence showing that a single microorganism that acts as a keystone pathogen can cause quantitative and qualitative alterations to the commensal microbiota, which in turn leads to dysbiosis.
16. Hajishengallis, G., Darveau, R. P. & Curtis, M. A. The keystone-pathogen hypothesis. *Nature Rev. Microbiol.* **10**, 717–725 (2012).
17. Darveau, R. P. Periodontitis: a polymicrobial disruption of host homeostasis. *Nature Rev. Microbiol.* **8**, 481–490 (2010).
18. Rosier, B. T., de Jager, M., Zaura, E. & Krom, B. P. Historical and contemporary hypotheses on the development of oral diseases: are we there yet? *Front Cell Infect. Microbiol.* **4**, 92 (2014).
19. Hajishengallis, G. & Lamont, R. J. Beyond the red complex and into more complexity: the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. *Mol. Oral Microbiol.* **27**, 409–419 (2012).
20. Hajishengallis, G. Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. *Trends Immunol.* **35**, 3–11 (2014).
21. Han, Y. W. *et al.* Term stillbirth caused by oral *Fusobacterium nucleatum*. *Obstet. Gynecol.* **115**, 442–445 (2010).
22. Heo, S. M., Haase, E. M., Lesse, A. J., Gill, S. R. & Scannapieco, F. A. Genetic relationships between respiratory pathogens isolated from dental plaque and bronchoalveolar lavage fluid from patients in the intensive care unit undergoing mechanical ventilation. *Clin. Infect. Dis.* **47**, 1562–1570 (2008).
23. Scannapieco, F. A. Individuals with chronic obstructive pulmonary disease (COPD) may be more likely to have more severe periodontal disease than individuals without COPD. *J. Evid. Based Dent. Pract.* **14**, 79–81 (2014).
24. Tan, L., Wang, H., Li, C. & Pan, Y. 16S rDNA-based metagenomic analysis of dental plaque and lung bacteria in patients with severe acute exacerbations of chronic obstructive pulmonary disease. *J. Periodont. Res.* **49**, 760–769 (2014).
25. Kozarov, E. V., Dorn, B. R., Shelburne, C. E., Dunn, W. A. Jr & Progulske-Fox, A. Human atherosclerotic plaque contains viable invasive *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *Arterioscler. Thromb. Vasc. Biol.* **25**, e17–e18 (2005).
26. Haraszthy, V. I., Zambon, J. J., Trevisan, M., Zeid, M. & Genco, R. J. Identification of periodontal pathogens in atheromatous plaques. *J. Periodontol.* **71**, 1554–1560 (2000).
27. Carrion, J. *et al.* Microbial carriage state of peripheral blood dendritic cells (DCs) in chronic periodontitis influences DC differentiation, atherogenic potential. *J. Immunol.* **189**, 3178–3187 (2012).
This study combines clinical and mechanistic evidence implicating blood myeloid DCs as transport vehicles for the systemic dissemination of *P. gingivalis* from its oral habitat.
28. Velsko, I. M. *et al.* Active invasion of oral and aortic tissues by *Porphyromonas gingivalis* in mice causally links periodontitis and atherosclerosis. *PLoS ONE* **9**, e97811 (2014).
29. Slocum, C. *et al.* Distinct lipid A moieties contribute to pathogen-induced site-specific vascular inflammation. *PLoS Pathog.* **10**, e1004215 (2014).
This study shows that the ability of *P. gingivalis* to enzymatically modify the lipid A moiety of its lipopolysaccharide allows it to escape from TLR4-mediated immunity and persist while inducing atherogenic inflammation at sites distant from the initial infection site.
30. Maresz, K. J. *et al.* *Porphyromonas gingivalis* facilitates the development and progression of destructive arthritis through its unique bacterial peptidylarginine deiminase (PAD). *PLoS Pathog.* **9**, e1003627 (2013).
This study was the first to provide an *in vivo* mechanistic link between *P. gingivalis* PPAD and rheumatoid arthritis associated with ACPAs.
31. Wegner, N. *et al.* Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and α-enolase: implications for autoimmunity in rheumatoid arthritis. *Arthritis Rheumatol.* **62**, 2662–2672 (2010).
32. Mikuls, T. R. *et al.* Periodontitis and *Porphyromonas gingivalis* in patients with rheumatoid arthritis. *Arthritis Rheumatol.* **66**, 1090–1100 (2014).
33. Koziel, J., Mydel, P. & Potempa, J. The link between periodontal disease and rheumatoid arthritis: an updated review. *Curr. Rheumatol. Rep.* **16**, 408 (2014).
34. Perez-Chaparro, P. J. *et al.* Newly identified pathogens associated with periodontitis: a systematic review. *J. Dent. Res.* **93**, 846–858 (2014).
35. Sima, C. & Glogauer, M. Neutrophil dysfunction and host susceptibility to periodontal inflammation: current state of knowledge. *Curr. Oral Health Rep.* **1**, 95–103 (2014).
36. Nussbaum, G. & Shapira, L. How has neutrophil research improved our understanding of periodontal pathogenesis? *J. Clin. Periodontol.* **38**, 49–59 (2011).
37. Yilmaz, O. The chronicles of *Porphyromonas gingivalis*: the microbium, the human oral epithelium and their interplay. *Microbiology* **154**, 2897–2903 (2008).
38. Darveau, R. P. *Porphyromonas gingivalis* neutrophil manipulation: risk factor for periodontitis? *Trends Microbiol.* **22**, 428–429 (2014).
39. Bostanci, N. & Belibasakis, G. N. *Porphyromonas gingivalis*: an invasive and evasive opportunistic oral pathogen. *FEMS Microbiol. Lett.* **333**, 1–9 (2012).
40. Holt, S. C., Ebersole, J., Felton, J., Brunsvold, M. & Kornman, K. S. Implantation of *Bacteroides gingivalis* in nonhuman primates initiates progression of periodontitis. *Science* **239**, 55–57 (1988).
41. Darveau, R. P., Hajishengallis, G. & Curtis, M. A. *Porphyromonas gingivalis* as a potential community activist for disease. *J. Dent. Res.* **91**, 816–820 (2012).
42. Maekawa, T. *et al.* *Porphyromonas gingivalis* manipulates complement and TLR signaling to uncouple bacterial clearance from inflammation and promote dysbiosis. *Cell Host Microbe* **15**, 768–778 (2014).
This investigation mechanistically dissects how periodontal bacteria can interfere with immune-mediated killing while promoting a nutritionally favourable inflammatory response, thereby perpetuating dysbiosis.
43. Hajishengallis, G. & Lamont, R. J. Breaking bad: manipulation of the host response by *Porphyromonas gingivalis*. *Eur. J. Immunol.* **44**, 328–338 (2014).
44. Jiao, Y. *et al.* Induction of bone loss by pathobiont-mediated nod1 signaling in the oral cavity. *Cell Host Microbe* **13**, 595–601 (2013).
This study demonstrates the concept of commensal-turned-pathobiont in periodontitis under conditions of disrupted tissue homeostasis.
45. Whitmore, S. E. & Lamont, R. J. The pathogenic persona of community-associated oral streptococci. *Mol. Microbiol.* **81**, 305–314 (2011).
46. Haffajee, A. D. *et al.* Subgingival microbiota in healthy, well-maintained elder and periodontitis subjects. *J. Clin. Periodontol.* **25**, 346–353 (1998).
47. Kesavalu, L. *et al.* Rat model of polymicrobial infection, immunity, and alveolar bone resorption in periodontal disease. *Infect. Immun.* **75**, 1704–1712 (2007).
48. Daep, C. A., Novak, E. A., Lamont, R. J. & Demuth, D. R. Structural dissection and *in vivo* effectiveness of a peptide inhibitor of *Porphyromonas gingivalis* adherence to *Streptococcus gordonii*. *Infect. Immun.* **79**, 67–74 (2011).
49. Orth, R. K., O'Brien-Simpson, N. M., Dashper, S. G. & Reynolds, E. C. Synergistic virulence of *Porphyromonas gingivalis* and *Treponema denticola* in a murine periodontitis model. *Mol. Oral Microbiol.* **26**, 229–240 (2011).
50. Polak, D. *et al.* Mouse model of experimental periodontitis induced by *Porphyromonas gingivalis/Fusobacterium nucleatum* infection: bone loss and host response. *J. Clin. Periodontol.* **36**, 406–410 (2009).
51. Tan, K. H. *et al.* *Porphyromonas gingivalis* and *Treponema denticola* exhibit metabolic symbioses. *PLoS Pathog.* **10**, e1003955 (2014).
52. Amano, A. *et al.* Genetic characteristics and pathogenic mechanisms of periodontal pathogens. *Adv. Dent. Res.* **26**, 15–22 (2014).
53. Kuboniwa, M. *et al.* Insights into the virulence of oral biofilms: discoveries from proteomics. *Expert Rev. Proteom.* **9**, 311–323 (2012).
54. Cyktor, J. C. & Turner, J. Interleukin-10 and immunity against prokaryotic and eukaryotic intracellular pathogens. *Infect. Immun.* **79**, 2964–2973 (2011).
55. Hajishengallis, G. The inflammophilic character of the periodontitis-associated microbiota. *Mol. Oral Microbiol.* **29**, 248–257 (2014).
56. Hajishengallis, E. & Hajishengallis, G. Neutrophil homeostasis and periodontal health in children and adults. *J. Dent. Res.* **93**, 231–237 (2014).
57. Moutsopoulos, N. M. *et al.* Defective neutrophil recruitment in leukocyte adhesion deficiency type I disease causes local IL-17–driven inflammatory bone loss. *Sci. Transl. Med.* **6**, 229ra240 (2014).
58. Ryder, M. I. Comparison of neutrophil functions in aggressive and chronic periodontitis. *Periodontol. 2000* **53**, 124–137 (2010).
59. Chapple, I. L. & Matthews, J. B. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontol. 2000* **43**, 160–232 (2007).
60. Eskan, M. A. *et al.* The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss. *Nature Immunol.* **13**, 465–473 (2012).
61. Lee, W., Aitken, S., Sodek, J. & McCulloch, C. A. Evidence of a direct relationship between neutrophil collagenase activity and periodontal tissue destruction *in vivo*: role of active enzyme in human periodontitis. *J. Periodon. Res.* **30**, 23–33 (1995).
62. Hernandez, M. *et al.* Associations between matrix metalloproteinase-8 and -14 and myeloperoxidase in gingival crevicular fluid from subjects with progressive chronic periodontitis: a longitudinal study. *J. Periodontol.* **81**, 1644–1652 (2010).
63. Landzberg, M., Doering, H., Aboodi, G. M., Tenenbaum, H. C. & Glogauer, M. Quantifying oral inflammatory load: oral neutrophil counts in periodontal health and disease. *J. Periodont. Res.* http://dx.doi.org/10.1111/jre.12211 (2014).

64. Ricklin, D., Hajishengallis, G., Yang, K. & Lambris, J. D. Complement: a key system for immune surveillance and homeostasis. *Nature Immunol.* **11**, 785–797 (2010).
65. Popadiak, K., Potempa, J., Riesbeck, K. & Blom, A. M. Biphasic effect of gingipains from *Porphyromonas gingivalis* on the human complement system. *J. Immunol.* **178**, 7242–7250 (2007).
66. Jusko, M. *et al.* A metalloproteinase karilysin present in the majority of *Tannerella forsythia* isolates inhibits all pathways of the complement system. *J. Immunol.* **188**, 2338–2349 (2012).
67. Potempa, M. *et al.* Interpain A, a cysteine proteinase from *Prevotella intermedia*, inhibits complement by degrading complement factor C3. *PLoS Pathog.* **5**, e1000316 (2009).
68. Miller, D. P. *et al.* Structure of factor H-binding protein B (FhbB) of the periopathogen, *Treponema denticola*: insights into progression of periodontal disease. *J. Biol. Chem.* **287**, 12715–12722 (2012).
69. Malm, S. *et al.* Acquisition of complement inhibitor serine protease factor I and its cofactors C4b-binding protein and factor H by *Prevotella intermedia*. *PLoS ONE* **7**, e34852 (2012).
70. Liang, S. *et al.* The C5a receptor impairs IL-12-dependent clearance of *Porphyromonas gingivalis* and is required for induction of periodontal bone loss. *J. Immunol.* **186**, 869–877 (2011).
71. Burns, E., Bachrach, G., Shapira, L. & Nussbaum, G. Cutting Edge: TLR2 is required for the innate response to *Porphyromonas gingivalis*: activation leads to bacterial persistence and TLR2 deficiency attenuates induced alveolar bone resorption. *J. Immunol.* **177**, 8296–8300 (2006).
72. Myneni, S. R. *et al.* TLR2 signaling and Th2 responses drive *Tannerella forsythia*-induced periodontal bone loss. *J. Immunol.* **187**, 501–509 (2011).
73. Shin, J. E., Baek, K. J., Choi, Y. S. & Choi, Y. A periodontal pathogen *Treponema denticola* hijacks the *Fusobacterium nucleatum*-driven host response. *Immunol. Cell Biol.* **91**, 503–510 (2013).
74. Wang, M. *et al.* Microbial hijacking of complement-toll-like receptor crosstalk. *Sci. Signal.* **3**, ra11 (2010).
75. Taxman, D. J. *et al. Porphyromonas gingivalis* mediates inflammasome repression in polymicrobial cultures through a novel mechanism involving reduced endocytosis. *J. Biol. Chem.* **287**, 32791–32799 (2012).
76. Lamkanfi, M. & Dixit, V. M. Mechanisms and functions of inflammasomes. *Cell* **157**, 1013–1022 (2014).
77. Moutsopoulos, N. M. *et al. Porphyromonas gingivalis* promotes Th17 inducing pathways in chronic periodontitis. *J. Autoimmun.* **39**, 294–303 (2012).
78. Jauregui, C. E. *et al.* Suppression of T-cell chemokines by *Porphyromonas gingivalis*. *Infect. Immun.* **81**, 2288–2295 (2013).
This study shows that the *P. gingivalis*-induced local 'chemokine paralysis', which was originally shown to affect innate immunity, also involves the suppression of T_H1-type chemokines.
79. Hayashi, C. *et al.* Protective role for TLR4 signaling in atherosclerosis progression as revealed by infection with a common oral pathogen. *J. Immunol.* **189**, 3681–3688 (2012).
80. Gemmell, E., Yamazaki, K. & Seymour, G. J. The role of T cells in periodontal disease: homeostasis and autoimmunity. *Periodontol. 2000* **43**, 14–40 (2007).
81. Gaffen, S. L. & Hajishengallis, G. A new inflammatory cytokine on the block: re-thinking periodontal disease and the Th1/Th2 paradigm in the context of Th17 cells and IL-17. *J. Dent. Res.* **87**, 817–828 (2008).
82. Bahekar, A. A., Singh, S., Saha, S., Molnar, J. & Arora, R. The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: a meta-analysis. *Am. Heart J.* **154**, 830–837 (2007).
83. Friedewald, V. E. *et al.* The American Journal of Cardiology and Journal of Periodontology Editors' Consensus: periodontitis and atherosclerotic cardiovascular disease. *Am. J. Cardiol.* **104**, 59–68 (2009).
84. Tonetti, M. S. *et al.* Treatment of periodontitis and endothelial function. *N. Engl. J. Med.* **356**, 911–920 (2007).
85. Offenbacher, S. *et al.* Results from the Periodontitis and Vascular Events (PAVE) Study: a pilot multicentered, randomized, controlled trial to study effects of periodontal therapy in a secondary prevention model of cardiovascular disease. *J. Periodontol.* **80**, 190–201 (2009).
86. Desvarieux, M. *et al.* Changes in clinical and microbiological periodontal profiles relate to progression of carotid intima-media thickness: the Oral Infections and Vascular Disease Epidemiology study. *J. Am. Heart Assoc.* **2**, e000254 (2013).
87. Brodala, N. *et al. Porphyromonas gingivalis* bacteremia induces coronary and aortic atherosclerosis in normocholesterolemic and hypercholesterolemic pigs. *Arterioscler Thromb. Vasc. Biol.* **25**, 1446–1451 (2005).
88. Tonetti, M. S. Periodontitis and risk for atherosclerosis: an update on intervention trials. *J. Clin. Periodontol.* **36** (Suppl. 10), 15–19 (2009).
89. Arimatsu, K. *et al.* Oral pathobiont induces systemic inflammation and metabolic changes associated with alteration of gut microbiota. *Sci. Rep.* **4**, 4828 (2014).
90. Gibson, F. C. 3rd *et al.* Innate immune recognition of invasive bacteria accelerates atherosclerosis in apolipoprotein E-deficient mice. *Circulation* **109**, 2801–2806 (2004).
91. Jain, A. *et al.* Role for periodontitis in the progression of lipid deposition in an animal model. *Infect. Immun.* **71**, 6012–6018 (2003).
92. Zelkha, S. A., Freilich, R. W. & Amar, S. Periodontal innate immune mechanisms relevant to atherosclerosis and obesity. *Periodontol. 2000* **54**, 207–221 (2010).
93. Chiu, B. Multiple infections in carotid atherosclerotic plaques. *Am. Heart J.* **138**, S534–S536 (1999).
94. Reyes, L., Herrera, D., Kozarov, E., Roldan, S. & Progulske-Fox, A. Periodontal bacterial invasion and infection: contribution to atherosclerotic pathology. *J. Clin. Periodontol.* **40** (Suppl. 14), 30–50 (2013).
95. Hayashi, C. *et al.* Pathogen-mediated inflammatory atherosclerosis is mediated in part via Toll-like receptor 2-induced inflammatory responses. *J. Innate Immun.* **2**, 334–343 (2010).
96. Coats, S. R. *et al.* Human Toll-like receptor 4 responses to *P. gingivalis* are regulated by lipid A 1- and 4′- phosphatase activities. *Cell. Microbiol.* **11**, 1587–1599 (2009).
97. Curtis, M. A. *et al.* Temperature dependent modulation of *Porphyromonas gingivalis* lipid A structure and interaction with the innate host defences. *Infect. Immun.* **79**, 1187–1193 (2011).
98. Al-Qutub, M. N. *et al.* Hemin-dependent modulation of the lipid A structure of *Porphyromonas gingivalis* lipopolysaccharide. *Infect. Immun.* **74**, 4474–4485 (2006).
References 97 and 98 show, at the molecular level, how environmental factors can influence microbial virulence and host–microorganism interactions.
99. Zenobia, C. *et al. Porphyromonas gingivalis* lipid A phosphatase activity is critical for colonization and increasing the commensal load in the rabbit ligature model. *Infect. Immun.* **82**, 650–659 (2014).
100. Coats, S. R., Do, C. T., Karimi-Naser, L. M., Braham, P. H. & Darveau, R. P. Antagonistic lipopolysaccharides block *E. coli* lipopolysaccharide function at human TLR4 via interaction with the human MD-2 lipopolysaccharide binding site. *Cell. Microbiol.* **9**, 1191–1202 (2007).
101. Bostanci, N. *et al. Porphyromonas gingivalis* antagonises *Campylobacter rectus* induced cytokine production by human monocytes. *Cytokine* **39**, 147–156 (2007).
102. Wang, M. *et al.* Fimbrial proteins of *Porphyromonas gingivalis* mediate *in vivo* virulence and exploit TLR2 and complement receptor 3 to persist in macrophages. *J. Immunol.* **179**, 2349–2358 (2007).
103. Wang, M. & Hajishengallis, G. Lipid raft-dependent uptake, signalling and intracellular fate of *Porphyromonas gingivalis* in mouse macrophages. *Cell. Microbiol.* **10**, 2029–2042 (2008).
104. Manes, S., del Real, G. & Martinez, A. C. Pathogens: raft hijackers. *Nature Rev. Immunol.* **3**, 557–568 (2003).
105. Zeituni, A. E., McCaig, W., Scisci, E., Thanassi, D. G. & Cutler, C. W. The native 67-kilodalton minor fimbria of *Porphyromonas gingivalis* is a novel glycoprotein with DC-SIGN-targeting motifs. *J. Bacteriol.* **192**, 4103–4110 (2010).
106. Zeituni, A. E., Jotwani, R., Carrion, J. & Cutler, C. W. Targeting of DC-SIGN on human dendritic cells by minor fimbriated *Porphyromonas gingivalis* strains elicits a distinct effector T cell response. *J. Immunol.* **183**, 5694–5704 (2009).
107. Miles, B. *et al.* Secondary lymphoid organ homing phenotype of human myeloid dendritic cells disrupted by an intracellular oral pathogen. *Infect. Immun.* **82**, 101–111 (2014).
108. Liang, Z. *et al.* Silencing of CXCR4 blocks breast cancer metastasis. *Cancer Res.* **65**, 967–971 (2005).
109. Sainz, J. & Sata, M. CXCR4, a key modulator of vascular progenitor cells. *Arterioscler. Thromb. Vasc. Biol.* **27**, 263–265 (2007).
110. Belstrom, D. *et al.* The atherogenic bacterium *Porphyromonas gingivalis* evades circulating phagocytes by adhering to erythrocytes. *Infect. Immun.* **79**, 1559–1565 (2011).
111. Madrigal, A. G., Barth, K., Papadopoulos, G. & Genco, C. A. Pathogen-mediated proteolysis of the cell death regulator RIPK1 and the host defense modulator RIPK2 in human aortic endothelial cells. *PLoS Pathog.* **8**, e1002723 (2012).
112. Huck, O., Elkaim, R., Davideau, J. L. & Tenenbaum, H. *Porphyromonas gingivalis*-impaired innate immune response via NLRP3 proteolysis in endothelial cells. *Innate Immun.* **21**, 65–72 (2014).
113. Harokopakis, E., Albzreh, M. H., Martin, M. H. & Hajishengallis, G. TLR2 transmodulates monocyte adhesion and transmigration via Rac1- and PI3K-mediated inside-out signaling in response to *Porphyromonas gingivalis* fimbriae. *J. Immunol.* **176**, 7645–7656 (2006).
114. Takahashi, Y. *et al.* Fimbria-dependent activation of pro-inflammatory molecules in *Porphyromonas gingivalis* infected human aortic endothelial cells. *Cell. Microbiol.* **8**, 738–757 (2006).
115. Jain, S., Coats, S. R., Chang, A. M. & Darveau, R. P. A novel class of lipoprotein lipase-sensitive molecules mediates Toll-like receptor 2 activation by *Porphyromonas gingivalis*. *Infect. Immun.* **81**, 1277–1286 (2013).
116. Clark, R. B. *et al.* Serine lipids of *Porphyromonas gingivalis* are human and mouse Toll-like receptor 2 ligands. *Infect. Immun.* **81**, 3479–3489 (2013).
117. Delbosc, S. *et al. Porphyromonas gingivalis* participates in pathogenesis of human abdominal aortic aneurysm by neutrophil activation. Proof of concept in rats. *PLoS ONE* **6**, e18679 (2011).
118. Nakayama, K. *Porphyromonas gingivalis* cell-induced hemagglutination and platelet aggregation. *Periodontol. 2000* **54**, 45–52 (2010).
119. Han, Y. W., Houcken, W., Loos, B. G., Schenkein, H. A. & Tezal, M. Periodontal disease, atherosclerosis, adverse pregnancy outcomes, and head-and-neck cancer. *Adv. Dent. Res.* **26**, 47–55 (2014).
120. Chen, Y. W. *et al.* Association between periodontitis and anti-cardiolipin antibodies in Buerger disease. *J. Clin. Periodontol.* **36**, 830–835 (2009).
121. Li, X. Y. *et al. Porphyromonas gingivalis* lipopolysaccharide increases lipid accumulation by affecting CD36 and ATP-binding cassette transporter A1 in macrophages. *Oncol. Rep.* **30**, 1329–1336 (2013).
122. Giacona, M. B. *et al. Porphyromonas gingivalis* induces its uptake by human macrophages and promotes foam cell formation *in vitro*. *FEMS Microbiol. Lett.* **241**, 95–101 (2004).
123. Sanz, M. & Kornman, K. Periodontitis and adverse pregnancy outcomes: consensus report of the Joint EFP/AAP workshop on periodontitis and systemic diseases. *J. Clin. Periodontol.* **40** (Suppl. 14), 164–169 (2013).
124. Liu, H., Redline, R. W. & Han, Y. W. *Fusobacterium nucleatum* induces fetal death in mice via stimulation of TLR4-mediated placental inflammatory response. *J. Immunol.* **179**, 2501–2508 (2007).
This was the first mechanistic study linking an oral pathogen to pregnancy complications in an animal model.
125. Han, Y. W. *et al. Fusobacterium nucleatum* induces premature and term stillbirths in pregnant mice: implication of oral bacteria in preterm birth. *Infect. Immun.* **72**, 2272–2279 (2004).
126. Schenkein, H. A. *et al.* Anti-cardiolipin antibodies in sera from patients with periodontitis. *J. Dent. Res.* **82**, 919–922 (2003).
127. Schenkein, H. A., Bradley, J. L. & Purkall, D. B. Anticardiolipin in *Porphyromonas gingivalis* antisera causes fetal loss in mice. *J. Dent. Res.* **92**, 814–818 (2013).
This report implicates molecular mimicry as a mechanism by which the host response to *P. gingivalis* can induce cardiolipin-specific autoantibodies that cause fetal loss in mice.
128. Scher, J. U., Bretz, W. A. & Abramson, S. B. Periodontal disease and subgingival microbiota as contributors for rheumatoid arthritis pathogenesis: modifiable risk factors? *Curr. Opin. Rheumatol.* **26**, 424–429 (2014).

129. Joseph, R., Rajappan, S., Nath, S. G. & Paul, B. J. Association between chronic periodontitis and rheumatoid arthritis: a hospital-based case-control study. *Rheumatol. Int.* **33**, 103–109 (2013).
130. de Pablo, P., Dietrich, T. & McAlindon, T. E. Association of periodontal disease and tooth loss with rheumatoid arthritis in the US population. *J. Rheumatol.* **35**, 70–76 (2008).
131. Suwannalai, P., Trouw, L. A., Toes, R. E. & Huizinga, T. W. Anti-citrullinated protein antibodies (ACPA) in early rheumatoid arthritis. *Mod. Rheumatol.* **22**, 15–20 (2012).
132. Gyorgy, B., Toth, E., Tarcsa, E., Falus, A. & Buzas, E. I. Citrullination: a posttranslational modification in health and disease. *Int. J. Biochem. Cell Biol.* **38**, 1662–1677 (2006).
133. Pyrc, K. *et al.* Inactivation of epidermal growth factor by *Porphyromonas gingivalis* as a potential mechanism for periodontal tissue damage. *Infect. Immun.* **81**, 55–64 (2013).
134. Pratesi, F. *et al.* HLA shared epitope and ACPA: just a marker or an active player? *Autoimmun. Rev.* **12**, 1182–1187 (2013).
135. Lundberg, K. *et al.* Antibodies to citrullinated α-enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase. *Arthritis Rheum.* **58**, 3009–3019 (2008).
This study identifies an immunodominant epitope in citrullinated α-enolase that is cross-reactive with citrullinated *P. gingivalis* enolase and is implicated in rheumatoid arthritis; hence, these findings are consistent with a role for *P. gingivalis* and its citrullinating enzyme in priming autoimmunity.
136. Catrina, A. I., Ytterberg, A. J., Reynisdottir, G., Malmstrom, V. & Klareskog, L. Lungs, joints and immunity against citrullinated proteins in rheumatoid arthritis. *Nature Rev. Rheumatol.* (2014).
137. Sokolove, J. *et al.* Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. *PLoS ONE* **7**, e35296 (2012).
138. Gully, N. *et al. Porphyromonas gingivalis* peptidylarginine deiminase, a key contributor in the pathogenesis of experimental periodontal disease and experimental arthritis. *PLoS ONE* **9**, e100838 (2014).
139. Paju, S. & Scannapieco, F. A. Oral biofilms, periodontitis, and pulmonary infections. *Oral Dis.* **13**, 508–512 (2007).
140. Finegold, S. M. Aspiration pneumonia. *Rev. Infect. Dis.* **13**, S737–S742 (1991).
141. Awano, S. *et al.* Oral health and mortality risk from pneumonia in the elderly. *J. Dent. Res.* **87**, 334–339 (2008).
142. Kimizuka, R., Kato, T., Ishihara, K. & Okuda, K. Mixed infections with *Porphyromonas gingivalis* and *Treponema denticola* cause excessive inflammatory responses in a mouse pneumonia model compared with monoinfections. *Microbes Infect.* **5**, 1357–1362 (2003).
143. Müller, F. Oral hygiene reduces the mortality from aspiration pneumonia in frail elders. *J. Dent. Res.* http://dx.doi.org/10.1177/0022034514552494 (2014).
144. Iinuma, T. *et al.* Denture wearing during sleep doubles the risk of pneumonia in the very elderly. *J. Dent. Res.* http://dx.doi.org/10.1177/0022034514552493 (2014).
145. Peter, K. P. *et al.* Association between periodontal disease and chronic obstructive pulmonary disease: a reality or just a dogma? *J. Periodontol.* **84**, 1717–1723 (2013).
146. Sethi, S. & Murphy, T. F. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. *N. Engl. J. Med.* **359**, 2355–2365 (2008).
147. Pan, Y., Teng, D., Burke, A. C., Haase, E. M. & Scannapieco, F. A. Oral bacteria modulate invasion and induction of apoptosis in HEp-2 cells by *Pseudomonas aeruginosa*. *Microb. Pathog.* **46**, 73–79 (2009).
148. Li, Q. *et al. Porphyromonas gingivalis* modulates *Pseudomonas aeruginosa*-induced apoptosis of respiratory epithelial cells through the STAT3 signaling pathway. *Microbes Infect.* **16**, 17–27 (2014).
149. Yao, L. *et al. Porphyromonas gingivalis* infection sequesters pro-apoptotic BAD through AKT in primary gingival epithelial cells. *Mol. Oral Microbiol.* **25**, 89–101 (2010).
150. Cannon, C. L., Kowalski, M. P., Stopak, K. S. & Pier, G. B. *Pseudomonas aeruginosa*-induced apoptosis is defective in respiratory epithelial cells expressing mutant cystic fibrosis transmembrane conductance regulator. *Am. J. Respir. Cell. Mol. Biol.* **29**, 188–197 (2003).
151. Papapanou, P. N. in *Oral Microbiology and Immunology* (eds Lamont, R. J., Hajishengallis, G. & Jenkinson, H.) 251–271 (ASM Press, 2014).
152. Kumar, P. S. Smoking and the subgingival ecosystem: a pathogen-enriched community. *Future Microbiol.* **7**, 917–919 (2012).
153. Stabholz, A., Soskolne, W. A. & Shapira, L. Genetic and environmental risk factors for chronic periodontitis and aggressive periodontitis. *Periodontol. 2000* **53**, 138–153 (2010).
154. Laine, M. L., Crielaard, W. & Loos, B. G. Genetic susceptibility to periodontitis. *Periodontol. 2000* **58**, 37–68 (2012).
155. Divaris, K. *et al.* Exploring the genetic basis of chronic periodontitis: a genome-wide association study. *Hum. Mol. Genet.* **22**, 2312–2324 (2013).
156. Bagaitkar, J. *et al.* Tobacco smoke augments *Porphyromonas gingivalis–Streptococcus gordonii* biofilm formation. *PLoS ONE* **6**, e27386 (2011).
157. Atanasova, K. R. & Yilmaz, O. Looking in the *Porphyromonas gingivalis* cabinet of curiosities: the microbium, the host and cancer association. *Mol. Oral Microbiol.* **29**, 55–66 (2014).
158. Castellarin, M. *et al. Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma. *Genome Res.* **22**, 299–306 (2012).
159. Rubinstein, M. R. *et al. Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. *Cell Host Microbe* **14**, 195–206 (2013).
This study generates both mechanistic and clinical evidence implicating the oral bacterium *F. nucleatum* in colorectal cancer.
160. Nagy, K. N., Sonkodi, I., Szoke, I., Nagy, E. & Newman, H. N. The microflora associated with human oral carcinomas. *Oral Oncol.* **34**, 304–308 (1998).
161. Katz, J., Onate, M. D., Pauley, K. M., Bhattacharyya, I. & Cha, S. Presence of *Porphyromonas gingivalis* in gingival squamous cell carcinoma. *Int. J. Oral Sci.* **3**, 209–215 (2011).
162. Ahn, J., Segers, S. & Hayes, R. B. Periodontal disease, *Porphyromonas gingivalis* serum antibody levels and orodigestive cancer mortality. *Carcinogenesis* **33**, 1055–1058 (2012).
163. Michaud, D. S. *et al.* Plasma antibodies to oral bacteria and risk of pancreatic cancer in a large European prospective cohort study. *Gut* **62**, 1764–1770 (2013).
164. Inaba, H. *et al. Porphyromonas gingivalis* promotes invasion of oral squamous cell carcinoma through induction of proMMP9 and its activation. *Cell. Microbiol.* **16**, 131–145 (2014).
165. Kuboniwa, M. *et al. P. gingivalis* accelerates gingival epithelial cell progression through the cell cycle. *Microbes Infect.* **10**, 122–128 (2008).
166. Mao, S. *et al.* Intrinsic apoptotic pathways of gingival epithelial cells modulated by *Porphyromonas gingivalis*. *Cell. Microbiol.* **9**, 1997–2007 (2007).
167. Yilmaz, O. *et al.* ATP scavenging by the intracellular pathogen *Porphyromonas gingivalis* inhibits P2X7-mediated host-cell apoptosis. *Cell. Microbiol.* **10**, 863–875 (2008).
168. Iwahori, K. *et al.* Overexpression of SOCS3 exhibits preclinical antitumor activity against malignant pleural mesothelioma. *Int. J. Cancer* **129**, 993–1005 (2011).
169. Moffatt, C. E. & Lamont, R. J. *Porphyromonas gingivalis* induction of microRNA-203 expression controls suppressor of cytokine signaling 3 in gingival epithelial cells. *Infect. Immun.* **79**, 2632–2637 (2011).
170. Groeger, S., Domann, E., Gonzales, J. R., Chakraborty, T. & Meyle, J. B7-H1 and B7-DC receptors of oral squamous carcinoma cells are upregulated by *Porphyromonas gingivalis*. *Immunobiology* **216**, 1302–1310 (2011).
171. Sznol, M. & Chen, L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. *Clin. Cancer Res.* **19**, 1021–1034 (2013).

Acknowledgements
The author's research is supported by grants from the US National Institutes of Health (DE015254, DE017138, DE021685 and AI068730). The author regrets that several studies could only be cited indirectly through comprehensive reviews, owing to space and reference number limitations.

Competing interests statement
The author declares no competing interests.