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Porphyromonas gingivalis: Immune subversion activities and role in periodontal dysbiosis

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Abstract

Purpose of review: This review summarizes mechanisms by which *Porphyromonas gingivalis* interacts with community members and the host so that it can persist in the periodontium under inflammatory conditions that drive periodontal disease.

Recent findings: Recent advances indicate that, in great part, the pathogenicity of *P. gingivalis* is dependent upon its ability to establish residence in the subgingival environment and to subvert innate immunity in a manner that uncouples the nutritionally favorable (for the bacteria) inflammatory response from antimicrobial pathways. While the initial establishment of *P. gingivalis* is dependent upon interactions with early colonizing bacteria, the immune subversion strategies of *P. gingivalis* in turn benefit co-habiting species.

Summary: Specific interspecies interactions and subversion of the host response contribute to the emergence and persistence of dysbiotic communities and are thus targets of therapeutic approaches for the treatment of periodontitis.

Keywords

P. gingivalis; inflammation; dysbiosis; immune subversion; periodontitis

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Conflict of Interest:

George Hajishengallis is an inventor of a patent that describes the use of complement inhibitors for therapeutic purposes in periodontal disease.

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Introduction

Perhaps the best-studied oral microorganism, *Porphyromonas gingivalis*, is a Gram-negative anaerobic and asaccharolytic bacterium that is implicated as a contributor in human periodontitis and is also suspected to play a role in systemic disorders, such as atherosclerosis, aspiration pneumonia, rheumatoid arthritis and Alzheimer's disease [1–9]. *P. gingivalis* expresses an array of virulence factors, such as cysteine proteinases (gingipains), lipopolysaccharide (LPS), hemagglutinins, and adhesive hair-like appendages known as fimbriae [10–14]. These molecules are thought to contribute to the ability of *P. gingivalis* to colonize, secure nutrients, and persist within the inflammatory environment of the periodontal pocket while evading host immunity [15, 16, 12, 17–21]. However, the potential pathogenicity of *P. gingivalis* is better appreciated through interactions with certain partner species and in the context of the greater microbial community. In this regard, recent advances from microbiome and mechanistic studies suggest a new model of periodontal disease pathogenesis, in which disease arises not from individual pathogens but from polymicrobial synergy and dysbiosis, which perturbs the ecologically balanced microbiota associated with periodontal health [22–31]. Here we review how *P. gingivalis*, as a community member, interacts with the host and other microbes leading to dysbiotic inflammation that contributes to periodontal disease development. This work is dedicated to Dr. Robert (Bob) J. Genco, a former mentor of one of the co-authors (GH), who is commemorated in this issue for his pivotal role in periodontal research and his inspiring influence on countless scientists in the field. Much of the progress achieved on the biology of *P. gingivalis* and its *fimA*-encoded fimbriae is based on pioneering studies by Bob and his group [32–41, 10].

P. gingivalis in heterotypic bacterial communities

The predominant habitat of *P. gingivalis* is in the subgingival crevice which develops into a periodontal pocket in periodontitis. Although *P. gingivalis* is indigenous to the oral cavity of humans, it can only be detected in a small fraction of periodontally healthy subjects. At a given time, *P. gingivalis* can be detected via a sensitive and specific PCR assay in 25% of subjects presenting with periodontal health or mild periodontitis (with pocket probing depths 5 mm or less). In contrast, it is detected in 79% of subjects with deep pockets [42]. The interactions with other microbial species in subgingival biofilms and the inflammatory products released by the host are likely to determine whether *P. gingivalis* can successfully establish in the subgingival environment.

The development of subgingival microbial communities is a complex process dependent upon stable attachment to the substratum, interspecies adhesive interactions, metabolic compatibility, and capacity of the community to resist host immunity while maintaining an inflammatory environment [31, 2, 43]. *P. gingivalis* depends on early colonizers, such as oral streptococci, to support its initial attachment and biofilm formation [44]. For instance, *P. gingivalis* engages in multivalent co-adherence interactions with *Streptococcus gordonii*, in which the major (FimA) and the minor (Mfa1) fimbrial subunit proteins of the former interact, respectively, with the surface proteins glyceraldehyde-3-phosphate dehydrogenase and SspA/B of the latter [45, 46]. Dual-species communities of *P. gingivalis* and *S. gordonii*

display mutualistic growth in in vitro models [47, 48]. Numerous interactions between co-adhered *S. gordonii* and *P. gingivalis* have been characterized including the elucidation that an *S. gordonii* gene, *cbe*, is essential for *P. gingivalis* accumulation on the streptococcal surface [49]. Later work revealed that the product of *cbe*, the folate precursor 4-aminobenzoate/para-amino benzoic acid (pABA) is responsible for enhancing *P. gingivalis* biofilm accumulation [50]. Exogenously added pABA was shown to alter the transcriptome and metabolome of *P. gingivalis*, increasing the expression of the adhesive fimbrial components FimA and Mfa1, among other effects. Consistent with this finding, pABA was seen to facilitate colonization of *P. gingivalis* in the mouse oral cavity. However, despite increased numbers, pABA-treated *P. gingivalis* induced significantly less bone loss than vehicle-treated cells. These findings show that the interaction of *P. gingivalis* and early-colonizing partners could modulate in a different manner colonization and pathogenicity.

P. gingivalis interactions with viridans-group streptococci may also be antagonistic. For instance, *Streptococcus cristatus*-derived arginine deiminase inhibits the expression of the FimA fimbrial protein in *P. gingivalis* leading to impaired periodontal colonization and pathogenicity of *P. gingivalis* in mice, consistent with observations that the distribution of *P. gingivalis* and *S. cristatus* is negatively correlated in the human subgingival biofilm [51–53]. Thus, distinct streptococcal species can potentially promote or suppress *P. gingivalis* colonization. Conversely, *P. gingivalis* has been shown to induce cell death and DNA fragmentation of the health-associated commensal *Streptococcus mitis*, although the mechanism behind this interaction is still unknown [54].

Other early colonizers and ubiquitous components of health-associated biofilms, such as *Veillonella* and *Fusobacterium* species, have been shown to interact synergistically with *P. gingivalis*. *Veillonella atypica* coaggregates with *P. gingivalis* via the *Veillonella* hemagglutinin protein Hag1 [55]. *Veillonella* species are also proposed to be important for the colonization of *P. gingivalis* as they possess the gene repertoire for de novo biosynthesis of heme, which is the iron form required by *P. gingivalis* for growth. Indeed, *V. atypica* supports the in vitro growth of *P. gingivalis* when heme is excluded from the growth medium and deletion of *hemE* in *V. atypica*, which abolishes heme synthesis, results in an abrogation of the growth-supporting effect [56]. Since *P. gingivalis* is an anaerobe, its colonization requires interactions with other community members able to reduce the environment to oxygen levels it can tolerate. *Fusobacterium nucleatum*, a ubiquitous oxygen-tolerant species has been shown to be important for the survival of other anaerobes, including *P. gingivalis*, in continuous culture chemostat models under aerated conditions [57, 58]. Although *F. nucleatum* is also an anaerobe, it possesses enzymatic activities such as that of NADH oxidase, which contribute to the rapid metabolism of oxygen and the reduction of the environment [57, 59]. In addition, *F. nucleatum* supports the growth of *P. gingivalis* by supplying it with carbon dioxide [57].

P. gingivalis engages in synergistic interactions with other pathogenic organisms in subgingival microbial communities. For example, isobutyric acid production by *P. gingivalis* stimulates the growth of *Treponema denticola*, and reciprocally *T. denticola* produces succinic acid that promotes *P. gingivalis* growth [60]. Moreover, upon contact with *T. denticola*, *P. gingivalis* upregulates the expression of adhesins and proteases [61]. Consistent

with these in vitro findings, *P. gingivalis* and *T. denticola* are synergistically pathogenic in vivo [62, 63]. With regard to cross-kingdom interactions, the internalin family surface protein InlJ of *P. gingivalis* binds to the candidal hyphal protein Als3 of *Candida albicans* leading to the upregulation of genes encoding components of the Type IX section system (T9SS) of *P. gingivalis* [64]. Given that important virulence factors of *P. gingivalis*, including its gingipains, are secreted through the T9SS, communities of *P. gingivalis* and *C. albicans* may exhibit increased pathogenicity. In this regard, *C. albicans* colonizes the periodontal pockets of approximately 15–20% of chronic periodontitis patients and its presence in periodontal pockets is associated with the severity of chronic periodontitis [65–68].

In vivo interactions are bound to be more complex than those elucidated using the reductionist model systems utilized by most of the studies described above. Simplified models, however, clearly show that the interaction of *P. gingivalis* with other subgingival species is a determinant of its colonization and pathogenic potential. While reductionist models do not capture the complex emergent characteristics of in vivo communities, they represent a proof of the concept that the community context plays a role in modulating the establishment of *P. gingivalis*.

P. gingivalis subversion of innate immunity

Progress in the past 20 years has increased our understanding of how *P. gingivalis* resists immune elimination and persists in periodontal tissues. It would not be possible to discuss all relevant research and the present review will focus primarily on how *P. gingivalis* manipulates Toll-like receptors (TLRs) and co-receptors as well as the complement system which intimately interacts with TLRs. Other evasive mechanisms of this oral pathogen, such as, to inhibit production of the IL-8 chemokine by gingival epithelial cells [69], to degrade secreted cytokines [70] as well as many other tactics have been covered in earlier reviews [71, 12, 72, 73].

TLRs, a major family of pattern-recognition receptors (PRRs), are strategically located at the host-microbe interface [74, 75]. They recognize conserved microbial structures known as microbe-associated molecular patterns (MAMPs) and play a central role in inducing innate immune responses for controlling infection [74, 75]. Different TLRs respond to distinct MAMPs (*e.g.*, TLR2 responds to lipoteichoic acid and TLR4 to LPS) thus endowing the innate immune response with relative specificity [76]. TLRs do not function in isolation but cooperate with other PRRs in multireceptor complexes within membrane lipid rafts [76–78]. In this regard, TLR4 alone is not sufficient for inducing a vigorous innate response to LPS and requires MD-2 and CD14 [79, 80]. TLR2 responds to its ligands in association with TLR1 or TLR6 as signaling partners, and with CD14 or CD36 as important coreceptors for robust activation of TLR2/1 or TLR2/6 complexes [76]. Moreover, TLRs engage in signaling crosstalk with the complement system, apparently to coordinate innate immunity and inflammation via either synergistic or antagonistic interactions [81, 82]. However, it seems that *P. gingivalis* has evolved mechanisms whereby it can exploit TLRs or their crosstalk interactions in a manner that increases its adaptive fitness as well as the pathogenicity (nososymbiocity) of the microbial community where it resides [83]. Indeed, *P. gingivalis* evades leukocyte killing by exploiting signaling crosstalk between TLRs and other

receptors, such as CXC-chemokine receptor 4 (CXCR4) [84–86], complement receptor 3 (CR3; Mac1) [87, 88], and complement C5a receptor 1 (C5aR1) [89–91]. Although first shown for *P. gingivalis*, some of these evasion mechanisms were later shown to be exploited by other pathogens, such as *Bacillus anthracis* and *Francisella tularensis* [92, 93] or even malignant tumors [94]. Strikingly, the manipulation of the periodontal host response by *P. gingivalis* benefits the entire microbial community, which becomes dysbiotic (altered composition and increased total counts) and causes inflammatory periodontal bone loss [26].

The ability of *P. gingivalis* to orchestrate experimental periodontitis in mice by promoting the emergence of a dysbiotic microbial community [91, 26], while being a quantitatively minor constituent of the microbiota has prompted its characterization as a keystone pathogen (Figure 1), by analogy to the crucial role of a keystone holding an entire arch together [26, 95, 27].

TLR4 antagonism—*P. gingivalis* expresses heterogeneous and atypical LPS molecules that act as potent TLR4 antagonists, weak agonists or are immunologically inert [96]. Specifically, *P. gingivalis* can enzymatically modify the lipid A moiety of its lipopolysaccharide and the shifting of lipid A activity from TLR4-agonistic to TLR4-antagonistic depends on endogenous lipid A phosphatases [96]. These enzymes are controlled by growth phase or environmental factors, such as temperature and hemin availability [97, 98]. Different lipid A structures produced by *P. gingivalis* include non-phosphorylated tetra-acylated lipid A (inert for TLR4 activation), mono-phosphorylated penta-acylated lipid A (weak TLR4 agonist), and mono-phosphorylated tetra-acylated lipid A (TLR4 antagonist). Genetic inactivation of 4′-phosphatase activity (or bacterial growth at ≥39°C) results in the synthesis of TLR4 agonist lipid A, while ablation of 1-phosphatase activity (or growth in hemin-replete conditions) results in TLR4 antagonist lipid A [96, 99]. The ability of this oral pathogen to manipulate the biological activity of its lipid A allows it to evade or proactively inhibit a variety of potential TLR4-mediated antimicrobial functions, such as inhibition of expression of antimicrobial peptides (β defensins) in human epithelial cells [28]. Moreover, the production of inert or antagonistic lipid A enhances the resistance of *P. gingivalis* to cationic antimicrobial peptides, owing to changes in the outer surface charge of the bacterial surface that affect the binding of cationic peptides [96, 97, 99, 100]. Since *P. gingivalis* releases LPS-bearing membrane vesicles that can readily diffuse in the crevice or even penetrate gingival tissue [13], the TLR4 antagonistic LPS of *P. gingivalis* might inhibit TLR4-dependent antimicrobial responses against other bacteria in the same mixed-species biofilm [28].

Exploitation of TLR2 and integrins—In contrast to TLR4, *P. gingivalis* does not appear to antagonize TLR2 at the receptor level. However, this oral bacterium can block TLR2 antimicrobial responses by inducing subversive signaling crosstalk between TLR2 and other receptors, such as, C5aR1, CXCR4 and the β2 integrin CD11b/CD18 (also known as Mac1 or complement receptor 3). This subsection will focus on CD11b/CD18. A major TLR2 ligand of *P. gingivalis* involved in these subversive interactions appears to be its *fimA* gene-encoded fimbriae. The fimbriae extend to a significant distance (up to 3 μm) from the bacterial cell wall [11], suggesting that they might be the first *P. gingivalis* molecule to

interact with host cells and initiate intracellular signaling. The interaction of *P. gingivalis* fimbriae with TLR2 is promoted by an initial binding event between the fimbriae and CD14, which greatly facilitates TLR2-induced NF-κB activation and production of proinflammatory cytokines [14, 101, 102] (Figure 2). In addition to this pro-inflammatory signaling pathway, the interaction of *P. gingivalis* and its fimbriae with CD14/TLR2 in monocytes/macrophages also stimulates proadhesive signaling that activates the ligand-binding capacity of the CD11b/CD18 integrin [103, 104] (Figure 2). The regulation of the binding affinity of β2 integrin from within the cell through proadhesive signaling is referred to as 'inside-out signaling' [105]. Inside-out signaling may target cytoplasmic proteins to the integrin cytoplasmic tails causing high-affinity conformational changes on the ligand-binding domains of integrins [105].

Following interaction with the CD14/TLR2 recognition complex, *P. gingivalis* FimA fimbriae induce inside-out signaling involving Rac1 phosphatidylinositol-3-kinase (PI3K), and cytohesin-1 that activates the ligand-binding capacity of CD11b/CD18 [103, 104, 106] (Figure 2). This mechanism was shown to promote CD11b/CD18-dependent monocyte adhesion and transendothelial migration [104]. Interestingly, however, *P. gingivalis* has co-opted this TLR2/CD11b/CD18 proadhesive pathway for CD11b/CD18 binding and entry into macrophages in a relatively safe manner that promotes the fitness of this pathogen [77] (Figure 2). Specifically, the interaction of CD11b/CD18 with *P. gingivalis* leads to suppression of bioactive IL-12, increased intracellular survival of the pathogen in macrophages, and enhanced in vivo persistence and capacity to cause periodontal bone loss [88, 87]. Therefore, CD11b/CD18 may represent an 'Achilles' heel' exploited by *P. gingivalis* to promote its persistence in the mammalian host, probably because CD11b/CD18 is not linked to vigorous microbicidal mechanisms [107–110].

Subversion of CXCR4-TLR2 crosstalk—The outcome of TLR2 stimulation by microbial pathogens may be influenced by differential association of TLR2 with co-receptors. In this regard, by means of its FimA fimbriae, which can induce co-association of CXCR4 with TLR2 in lipid rafts, *P. gingivalis* induces CXCR4/TLR2 signaling crosstalk in human monocytes or mouse macrophages that compromises their killing function [84]. Specifically, the *P. gingivalis*-induced crosstalk between CXCR4 and TLR2 leads to enhanced cAMP-dependent protein kinase A (PKA) signaling, which in turn suppresses the generation of nitric oxide, a potent antimicrobial molecule for intracellular bacterial killing [84] (Figure 2). Consistent with this mechanism, mice subjected to *P. gingivalis*-induced periodontitis and treated with a CXCR4 antagonist are protected against periodontal tissue colonization by *P. gingivalis* and development of periodontal disease [86]. Intriguingly, it was subsequently shown that the soluble protein pancreatic adenocarcinoma upregulated factor (PAUF) also interacts with the CXCR4-TLR2 complex and elevates intracellular cAMP levels for inhibiting cellular activation [94]. The fact that both *P. gingivalis* fimbriae and PAUF induce the formation of the same receptor complex and exploit it to control cell signaling [84, 94] suggests that microbes and tumors may share common immune-evasive strategies.

Manipulation of TLR2 and complement—An intriguing question has been how *P. gingivalis* manages to selectively inhibit immune elimination without overall inhibiting inflammation. This is a crucial issue: If *P. gingivalis* would simply cause immune suppression (as many other pathogens do in other parts of the body [111]), this would spare *P. gingivalis* from immune clearance but it would starve it to death. This is because the survival and growth of this asaccharolytic organism (and other periodontitis-associated bacteria) critically depends on inflammatory tissue breakdown products (*e.g.*, degraded proteins and hemin, a source of essential iron) [112, 2]. Since neutrophils are heavily involved in human periodontitis and comprise ≥95% of total leukocytes in the gingival crevice (or periodontal pocket), where they constantly encounter *P. gingivalis* [113–116], this question was addressed in the context of *P. gingivalis*-neutrophil interactions. The gingival crevicular neutrophils form what looks like a “defense wall” against the periodontal bacteria; however, in periodontitis, the neutrophils largely fail to control the bacteria despite being viable and capable of immune and inflammatory responses [114, 117–122]. At least one major mechanism by which *P. gingivalis* can manipulate neutrophil responses is via instigating a subversive complement-TLR cross-talk.

In this regard, the arginine-specific gingipains (HRgpA, RgpB) of *P. gingivalis* can cleave C5 to generate high local concentrations of biologically active C5a [123, 89] independently of the immunological activation of the complement system [124]. Thus, given that neutrophils can recognize *P. gingivalis* via TLR2 [125], this oral bacterium can coactivate C5aR1 and TLR2 [91]. *P. gingivalis*-induced C5aR1-TLR2 crosstalk signaling leads to ubiquitylation and proteasomal degradation of the TLR2 adapter MyD88, thereby preventing a host-protective antimicrobial response [91] (Figure 3). Furthermore, the C5aR1-TLR2 crosstalk induces the activation of Pl3K, which inhibits RhoA GTPase and actin polymerization and consequently the phagocytic uptake of *P. gingivalis* (as well as bystander bacteria) (Figure 3). Importantly, moreover, this TLR2-PI3K signaling pathway induces inflammation that is nutritionally beneficial to the bacteria. Therefore, *P. gingivalis* subverts intracellular signaling in human or mouse neutrophils in ways that uncouple inflammation from bactericidal activity. Importantly, the local pharmacological inhibition of C5aR1, TLR2 or PI3K in the gingiva of *P. gingivalis*-colonized mice leads to immune clearance of *P. gingivalis*, reverses microbial dysbiosis effected by *P. gingivalis* colonization and suppresses periodontal inflammation [91]. These findings not only are consistent with the keystone pathogen concept but suggested that complement inhibition may be an effective strategy to inhibit periodontal disease, especially since complement is required for a persisting periodontal inflammatory response (mice deficient in the central complement component C3 are protected from inflammatory bone loss) [126]. Indeed, local complement inhibition in non-human primates, using the C3 inhibitor Cp40 (AMY-101), protected the animals against inducible or naturally-occurring periodontal disease [127, 126, 128]. In 2019, AMY-101 received Investigational New Drug approval by the U.S. Food and Drug Administration for the first clinical trial to assess its efficacy in humans with periodontal inflammation (gingivitis) (ClinicalTrials.gov Identifier: NCT03694444).

Summary and conclusions

P. gingivalis requires interactions with other subgingival species, which are likely to represent the first determinants of its successful establishment in the subgingival environment. Once *P. gingivalis* stably colonizes a site, it interacts with the host creating favorable conditions for its long-term persistence. In so doing, *P. gingivalis* can favorably promote the greater microbial community. Although *P. gingivalis* can influence gene and protein expression in other community members by direct interbacterial interactions [54, 43, 72], the major keystone-related function of this oral organism is likely via interference with innate immunity (Figs. 1–3). This interference not only compromises the ability of the host to control the periodontal microbial community but also leads to a dysregulated inflammatory response that contributes to tissue damage and bone resorption. Tissue destruction will also release peptides and heme-containing compounds which stimulate the growth of *P. gingivalis* and bystander species in the community. Although based on studies in the mouse model, the keystone pathogen concept is also consistent with *P. gingivalis* often being a quantitatively minor constituent of human periodontitis-associated biofilms, despite its increased prevalence and association with progressive bone loss in periodontal patients [129–133, 22]. However, an intervention study targeting specifically *P. gingivalis* would be required to test whether *P. gingivalis* exerts keystone function in human periodontitis. In this regard, in non-human primates, in which *P. gingivalis* is a natural member of their periodontal microbiota, a gingipain-based vaccine was shown to cause reduction in both the counts of *P. gingivalis* and the total subgingival bacterial burden [134], suggesting that the entire microbial community benefits from the presence of *P. gingivalis*. The discussed literature suggests that therapeutic targeting of host immune responses may be an effective way to control *P. gingivalis* and periodontal disease. In this regard, anti-inflammatory approaches, such as complement inhibition [127, 126, 135], to block harmful inflammation would also have indirect antimicrobial effects (limitation of inflammatory exudate-derived nutrients for the bacteria). Moreover, targeting complement pathways in the gingival tissues could render the host non-responsive to the subversive action of *P. gingivalis*, thereby neutralizing its promoting effect on periodontitis.

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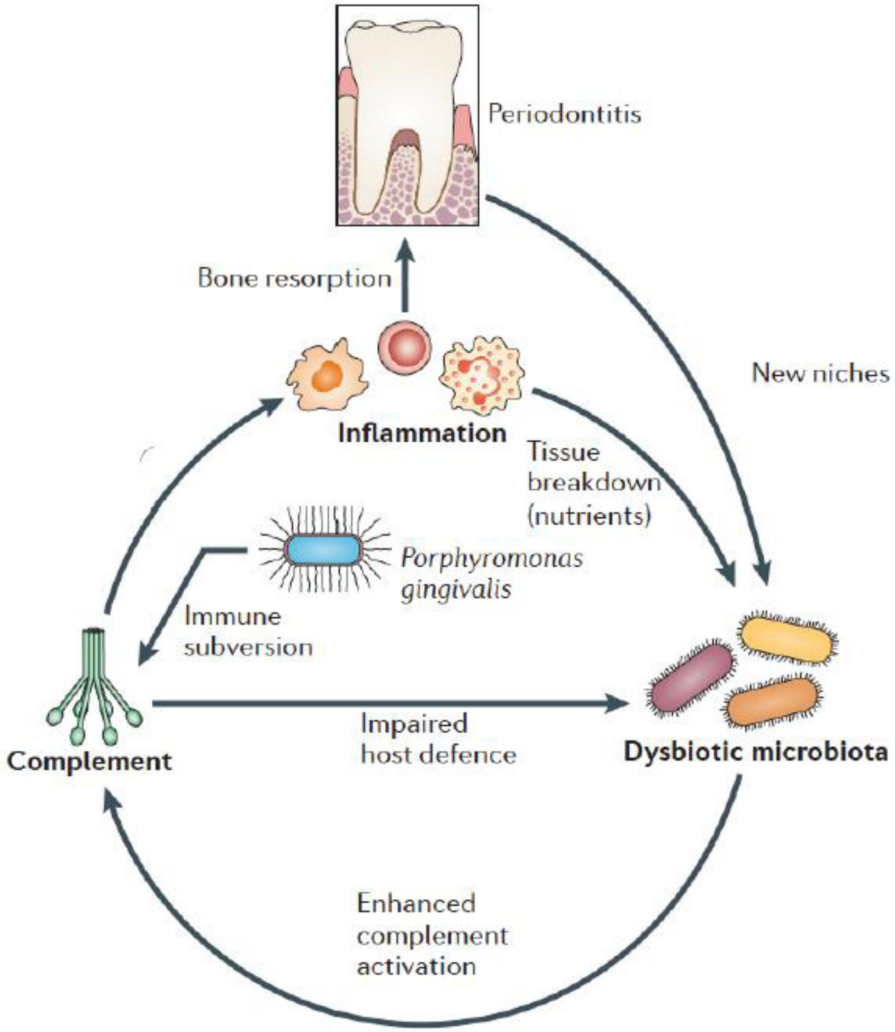

Figure 1. Keystone pathogen-induced dysbiosis in periodontal disease.

P. gingivalis subverts complement in a manner that compromises antimicrobial defense while enhancing inflammation. These effects contribute to dysbiotic changes of the periodontal microbiota (altered composition, increased total counts), which causes further inflammation, in great part through complement activation. Inflammatory tissue destruction fuels further bacterial growth by generating a nutrient-rich gingival inflammatory exudate (containing degraded host proteins and hemin, sources of amino acids and iron, respectively). These environmental changes favor proteolytic and asaccharolytic bacteria, thus explaining, at least in part, why inflammation causes compositional changes in the bacterial community. Moreover, inflammatory bone loss provides new niches for colonization by the dysbiotic microbiota. Overall, these changes create a self-sustained 'vicious cycle', where inflammation and dysbiosis are reciprocally reinforced. It should be noted, however, that whereas *P. gingivalis* can initiate dysbiosis, it is not an obligatory condition for dysbiosis. From reference [27]. Used by permission.

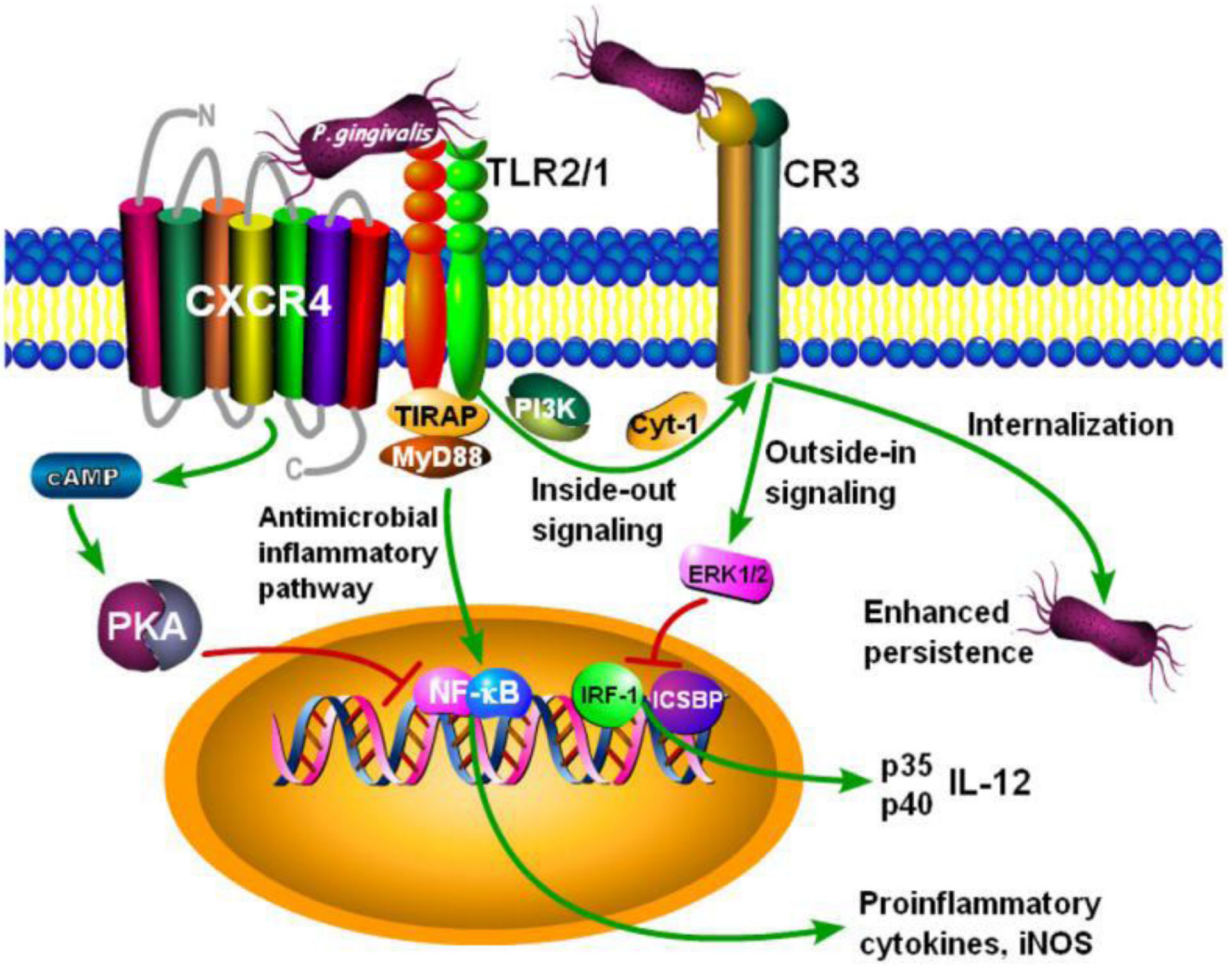

Figure 2. *P. gingivalis* instigates TLR2-CXCR4 and TLR2-CD11b/CD18 crosstalk interactions to subvert macrophages.

Through its FimA fimbriae, *P. gingivalis* can bind TLR2 (specifically the TLR2/TLR1 heterodimer; TLR2/1). Through a different structural component of the same molecule, *P. gingivalis* interacts with CXCR4 which cross-talks with and inhibits the TLR2/1-induced TIRAP/MyD88-mediated antimicrobial pathway. The underlying mechanism involves CXCR4-mediated stimulation of cAMP-dependent protein kinase A (PKA) signaling which limits NF-κB activation and induction of the inducible nitric oxide synthase (iNOS) that generates nitric oxide. The inhibitory effect on the production of nitric oxide, a potent antimicrobial mechanism for intracellular killing, promotes *P. gingivalis* survival in vitro and in vivo. By activating TLR2/1, *P. gingivalis* initiates inside-out signaling, which proceeds via phosphatidylinositol 3-kinase (PI3K) and cytohesin-1 to induce the high-affinity conformation of CD11b/CD18 (a β2 integrin also known as complement receptor 3). *P. gingivalis* binds activated CD11b/CD18 and is thereby internalized in a relatively safe manner as CD11b/CD18 is not linked to potent microbicidal mechanisms. Moreover, the *P. gingivalis*-CD11b/CD18 interaction activates extracellular signal-related kinase 1/2 (ERK1/2) signaling which downregulates IL-12 p35 and p40 mRNA expression. From reference [16]. Used by permission.

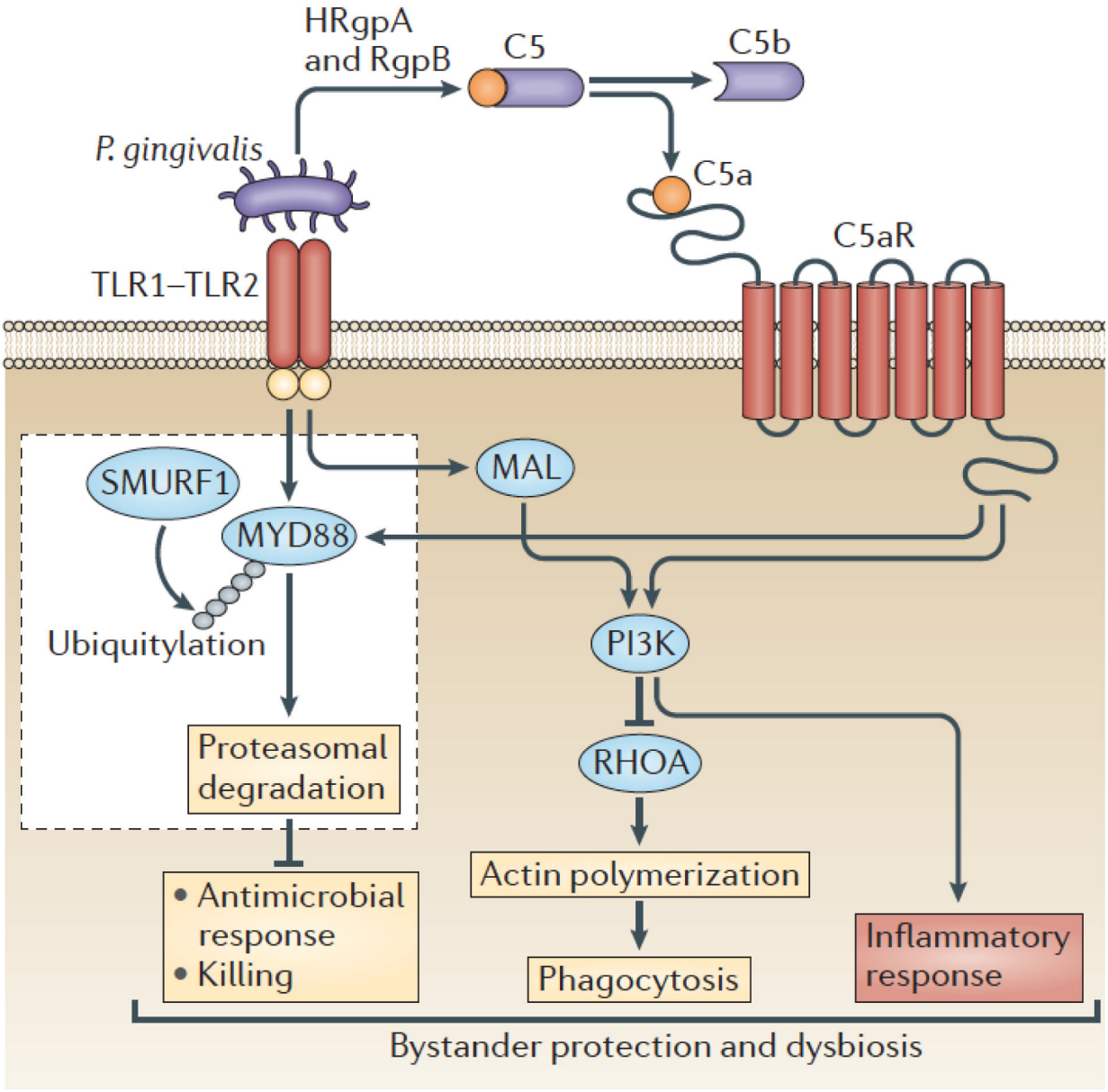

Figure 3. *P. gingivalis* dissociates immune clearance from inflammatory responses in neutrophils.

P. gingivalis activates the TLR1-TLR2 heterodimer and C5aR1, the latter through the generation of C5a by its gingipains. Specifically, these enzymes (HRgpA and RgpB) can cleave C5 to release biologically active C5a. The co-activation of TLR2 and C5aR1 by *P. gingivalis* and the resulting signaling crosstalk leads to the ubiquitylation (via SMURF, an E3 ubiquitin-protein ligase) and proteasomal degradation of the TLR2 adaptor MYD88, thereby blocking a host-protective antimicrobial mechanism. Moreover, the TLR2/C5aR1 crosstalk activates PI3K, which limits phagocytosis through the inhibition of the GTPase RHOA and hence actin polymerization. On the other hand, PI3K stimulates the production of inflammatory cytokines. Contrary to MYD88, another TLR2 adaptor, MYD88-like adaptor protein (MAL), participates in immune subversion by acting upstream of PI3K. These functionally integrated pathways offer 'bystander' protection to otherwise susceptible

periodontal organisms and enhance polymicrobial dysbiotic inflammation in vivo. From reference [6]. Used by permission.