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# The inflammophilic character of the periodontitis-associated microbiota

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## SUMMARY

In periodontitis, dysbiotic microbial communities exhibit synergistic interactions for enhanced protection from host defenses, nutrient acquisition, and persistence in an inflammatory environment. This Review discusses evidence that periodontitis-associated communities are 'inflammo-*philic*' (= loving or attracted to inflammation) in that they have evolved to not only endure inflammation but also to take advantage of it. In this regard, inflammation can drive the selection and enrichment of these pathogenic communities by providing a source of nutrients in the form of tissue breakdown products (*e.g*., degraded collagen peptides and heme-containing compounds). In contrast, those species that cannot benefit from the altered ecological conditions of the inflammatory environment, or for which host inflammation is detrimental, are likely to be outcompeted. Consistent with the concept that inflammation fosters the growth of dysbiotic microbial communities, the bacterial biomass of human periodontitis-associated biofilms was shown to increase with increasing periodontal inflammation. Conversely, anti-inflammatory treatments in animal models of periodontitis were shown to diminish the periodontal bacterial load, in addition to protecting from bone loss. The selective flourishing of inflammophilic bacteria can perpetuate inflammatory tissue destruction by setting off a 'vicious cycle' for disease progression, in which dysbiosis and inflammation reinforce each other. Therefore, the control of inflammation appears to be central to the treatment of periodontitis, as it is likely to control both dysbiosis and disease progression.

## INTRODUCTION

Periodontitis is a biofilm-induced chronic inflammatory disease characterized by loss of bone support of the dentition (Darveau, 2010; Hajishengallis, 2014b). The tooth-associated biofilm ('dental plaque') is required but is not sufficient by itself to induce periodontitis. In this regard, it is the host inflammatory response to this microbial challenge that primarily and ultimately causes the degradation of the periodontium, *i.e*., the tooth-supporting structures such as the gingiva and the underlying alveolar bone (Hajishengallis, 2014b). The precise mechanisms of periodontal pathogenesis are incompletely understood but disease initiation and progression invariably involves the breakdown of periodontal host-microbe homeostasis (Darveau, 2010; Hajishengallis, 2014b).

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Periodontal homeostasis can be disrupted by a variety of host- or microbe-related factors. These likely include congenital or acquired host immunodeficiencies, immunoregulatory defects associated with mutations or polymorphisms, old age, systemic diseases such as diabetes, obesity, environmental factors (*e.g*., smoking, diet, and stress), epigenetic modifications in response to environmental changes, and the presence of keystone pathogens that can transform a symbiotic microbiota into a dysbiotic one (Divaris *et al*., 2013; Eskan *et al*., 2012; Hajishengallis, 2014a; Hajishengallis *et al*., 2012; Laine *et al*., 2012; Lindroth & Park, 2013; Stabholz *et al*., 2010; Zhou *et al*., 2011). These factors could act alone or–more likely– in combination to cause dysbiosis of the periodontal microbiota and progression to periodontitis.

On the basis of evidence from human and animal studies discussed below, this Review proposes that the periodontitis-associated microbiota is inflammophilic (from the Greek suffix *philic*, meaning attracted to or loving) in that this microbial community has evolved to not only endure inflammation but also to exploit it to promote its adaptive fitness. Specifically, the available evidence is consistent with the notion that the inflamed periodontal pocket is conducive for the creation of a nutritionally rich environment that is permissive for bacterial outgrowth and pathogenesis.

## Recent advances in periodontal disease pathogenesis: Keystone pathogens and the PSD model

Recent independent studies employing metagenomic, metatranscriptomic, or mechanistic approaches collectively suggest that periodontitis is not caused by a select few bacteria, historically referred to as ‘periopathogens’ (Abusleme *et al*., 2013; Dewhirst *et al*., 2010; Duran-Pinedo *et al*., 2014; Griffen *et al*., 2012; Hajishengallis *et al*., 2011; Jiao *et al*., 2013; Jorth *et al*., 2014; Orth *et al*., 2011). In this respect, it has been proposed that periodontal disease pathogenesis involves polymicrobial synergy and dysbiosis (‘PSD model’) (Hajishengallis & Lamont, 2012, 2014), a notion consistent with the above-cited human meta-genomic/transcriptomic studies, as well as mechanistic studies in animal models and *in vitro* (Hajishengallis *et al*., 2011; Kesavalu *et al*., 2007; Maekawa *et al*., 2014b; Polak *et al*., 2009; Ramsey *et al*., 2011; Settem *et al*., 2012; Tan *et al*., 2014). According to the PSD model, periodontitis-associated microbial communities show synergistic interactions for enhanced colonization, nutrient procurement, and persistence in an inflammatory environment. The dysbiosis of the periodontal microbiota signifies an imbalance in the relative abundance or influence of microbial species within the ecosystem (as compared to health), leading to alterations in the host-microbial crosstalk sufficient to mediate destructive inflammation and bone loss (Abusleme *et al*., 2013; Hajishengallis, 2014b; Hajishengallis & Lamont, 2012). The recent metagenomic studies indicate that the periodontitis-associated microbiota is more heterogeneous and diverse than previously thought (*i.e*., on the basis of cultural studies) and many of the newly recognized organisms (*e.g*., *Filifactor alocis* and other species from the genera *Peptostreptococcaceae, Desulfobulbus, and Synergistetes*) show as good or better a correlation with disease than the classical red complex bacteria, *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* (Abusleme *et al*., 2013; Curtis *et al*., 2011; Dewhirst *et al*., 2010; Griffen *et al*., 2012; Griffen *et al*., 2011;

Kumar *et al*., 2006). Moreover, a plethora of virulence factors upregulated in the microbiome of periodontitis patients is primarily derived from species that are not traditionally considered as 'periopathogens' (Duran-Pinedo *et al*., 2014). Furthermore, the presence of the three red complex species in a complex subgingival biofilm model hardly affected the transcriptional profiling of challenged human gingival fibroblasts (Belibasakis *et al*., 2014). The above should not be interpreted to mean that the red complex bacteria do not play important roles in periodontitis. Simply, their role needs to be re-interpreted in the face of newly emerging evidence.

For instance, *P. gingivalis* was until recently thought to directly cause periodontitis, a notion consistent with its ability to cause bone loss upon its implantation in the oral cavity of animals, including mice and non-human primates (Baker *et al*., 1999; Holt *et al*., 1988). However, it was recently shown that *P. gingivalis* by itself fails to cause periodontitis in mice (*i.e*., germ-free mice that lack commensal bacteria). The actual role of *P. gingivalis* involves its ability to initiate the conversion from a symbiotic community structure to a dysbiotic one capable of causing destructive inflammation (Hajishengallis *et al*., 2011). In this regard, *P. gingivalis* expresses a variety of virulence factors (such as gingipains, atypical lipid A structures, and serine phosphatases), which manipulate the host response in ways that create a permissive environment for the growth of both *P. gingivalis* and bacteria co-habiting the same niche (Hajishengallis & Lamont, 2014; Hajishengallis *et al*., 2011; Takeuchi *et al*., 2013; Zenobia *et al*., 2014). This community-wide impact and the fact that *P. gingivalis* is a quantitatively minor constituent of the microbiota, has prompted its characterization as a keystone pathogen, by analogy to the crucial role of the literal keystone holding an entire arch together (Darveau *et al*., 2012; Hajishengallis *et al*., 2012). It should be noted that certain other periodontal species (*e.g*., *T. forsythia*, *T. denticola*, and *Aggregatibacter actinomycetemcomitans*) have also been shown to subvert the host response in ways that compromize protective immunity (Jusko *et al*., 2012; Miller *et al*., 2012; Potempa & Pike, 2009; Venketaraman *et al*., 2008). Whether these or other species can also play keystone roles (in similar or different environments or forms of periodontal disease) has not been addressed experimentally yet but it is a plausible hypothesis.

Although originally established in the mouse model, the keystone-pathogen concept in periodontitis is consistent with observations in other animal models and in humans: In rabbits, *P. gingivalis* causes a shift to a more anaerobic flora and an overall increase in the bacterial load of the dental biofilm (Hasturk *et al*., 2007; Zenobia *et al*., 2014). In non-human primates, where *P. gingivalis* is a natural inhabitant of the periodontal biofilm, a gingipain-based vaccine caused a reduction both in *P. gingivalis* numbers and in the total subgingival bacterial load (Page *et al*., 2007), suggesting that the presence of *P. gingivalis* benefits the entire biofilm. The keystone-pathogen concept is consistent with *P. gingivalis* also being a quantitatively minor constituent of human periodontitis-associated biofilms, despite its increased prevalence and association with progressive bone loss in periodontal patients (Abusleme *et al*., 2013; Chaves *et al*., 2000; Doungudomdacha *et al*., 2000; Kumar *et al*., 2006; Moore *et al*., 1982; Moore *et al*., 1991). *P. gingivalis* may additionally be detected, albeit with decreased frequency, in periodontally healthy individuals (Abusleme *et al*., 2013; Haffajee *et al*., 1998). The most likely explanations, which are not mutually

exclusive, involve alterations in the status of the host or the bacterium, such as strain and virulence diversity within the population structure of *P. gingivalis* (Darveau *et al*., 2012). From the host point of view, there may be individuals who can either resist or tolerate the conversion of the periodontal microbiota from a symbiotic to a dysbiotic state, by virtue of their intrinsic immuno-inflammatory status (*e.g*., hyporesponsive or lack-of-function polymorphisms that attenuate inflammation or microbial immune subversion). This notion is consistent with human genetic variability in innate immunity and susceptibility to periodontitis and with cases of individuals who remain periodontally healthy despite massive accumulation of dental plaque at dentogingival sites (Kinane *et al*., 2007; Kinane & Hart, 2003; Laine *et al*., 2012; Socransky & Haffajee, 1994).

It has been proposed that dysbiosis is not dependent so much on the particular microbial roster but rather on the specific gene combinations or collective virulence activity within the microbial community (Hajishengallis & Lamont, 2012). This notion is supported by a recent study that employed gene expression profiling to characterize patient-matched healthy and disease-associated periodontal microbiotas. This investigation showed that disease-associated microbial communities exhibit highly conserved metabolic gene expression profiles, despite high interpatient variability in terms of microbial composition (Jorth *et al*., 2014). Moreover, this concept and the fact that periodontitis is not uniquely a human disease (Page & Schroeder, 1982) further validates the use of appropriate animal models to study periodontal disease pathogenesis (Graves *et al*., 2008). The evidence in support of the inflammophilic character of the periodontitis-associated microbiota is based on both animal models and clinical observations in human patients (see below).

## Inflammation can drive the selection and enrichment of dysbiotic communities

The ecological succession from health to periodontitis reveals the emergence of newly-dominant community members rather than the appearance of novel species (Abusleme *et al*., 2013). This important recent finding is consistent with the ecologic plaque hypothesis proposed more than ten years ago (Marsh, 2003). According to this hypothesis, ‘periodontal pathogens’ are members of the normal microbiota but at levels too low to cause disease, whereas changes in ecologic conditions could favor the outgrowth of such organisms beyond a threshold sufficient to lead to periodontitis (Marsh, 2003). This threshold could be numerical or physiological, or a combination of both. In other words, this notion views periodontitis as an ecologic catastrophe. It cannot be overstated that periodontal inflammation is not simply a mechanism for host tissue destruction; from a microbe-centric standpoint, inflammation is an important source of nutrients and, therefore, can exert a powerful influence in the composition and numbers of the periodontal microbiota favoring those species that can both endure inflammation and utilize tissue breakdown products (Fig. 1). Stated in lay terms, bacteria do not cause inflammation to destroy the periodontal tissue but to obtain their food. Destructive inflammation generates abundant tissue-breakdown products that serve the nutritional needs of certain types of bacteria; for instance, degraded collagen and heme-containing compounds (haptoglobin, hemopexin, and hemoglobin) can be utilized by proteolytic and asaccharolytic bacteria to obtain essential amino-acids and

iron (Fig. 1) (Hajishengallis *et al*., 2012). Subgingival sites harboring periodontitis-associated bacteria exhibit a low redox potential and are bathed in gingival crevicular fluid (GCF), a nutritionally rich serum-like exudate, the flow of which increases with increasing periodontal inflammation (Marsh, 2003). These conditions support a microbial community with higher proportions of obligately anaerobic bacteria that thrive at the expense of aerobic species or those that cannot take advantage of the inflammatory environment. Indeed, those species that cannot benefit from these environmental changes, or for which host inflammation is detrimental, may have a fitness disadvantage and hence be outcompeted. The selective flourishing of inflammophilic bacteria acting as pathobionts can potentially perpetuate a self-feeding “vicious cycle” for incessant tissue destruction and bacterial overgrowth (Fig. 1) (Hajishengallis *et al*., 2012).

As alluded to above, *P. gingivalis* is an exemplar of immune subversive activity in the periodontal tissue (Bostanci & Belibasakis, 2012; Hajishengallis & Lambris, 2011; Yilmaz, 2008). This microbe is now thought to orchestrate rather than to directly cause inflammatory bone loss, which is largely mediated by pathobionts, *i.e*., commensals that under conditions of disrupted homeostasis have the potential to cause disease. *P. gingivalis* and other immune-subversive organisms, including *T. forsythia*, *T. denticola*, and *Prevotella intermedia*, interact with complement in complex ways including both inhibitory and stimulatory effects (Jusko *et al*., 2012; Krauss *et al*., 2010; Miller *et al*., 2012; Popadiak *et al*., 2007; Potempa & Pike, 2009). On the one hand, periodontal bacteria need to escape immune elimination, whereas, on the other hand, they have to proactively induce inflammation and thereby stimulate the flow of GCF to obtain essential nutrients (Hajishengallis *et al*., 2012; Hasturk *et al*., 2012). In other words, although immunosuppression is an immune evasion strategy used by many microbial pathogens, it is not a viable option for inflammophilic bacteria. In this context, *P. gingivalis* was shown to block a host-protective TLR2-MyD88 pathway in neutrophils via proteasomal degradation of MyD88, whereas it activates a proinflammatory TLR2MalPI3K pathway that also blocks phagocytosis (Maekawa *et al*., 2014b). Both subversive pathways require an intimate crosstalk between TLR2 and C5aR instigated by *P. gingivalis* (Fig. 2). The integrated mechanism mediates ‘bystander’ protection to otherwise susceptible bacteria and promotes dysbiotic inflammation *in vivo* (Maekawa *et al*., 2014b). This study has therefore dissected a mechanism by which *P. gingivalis* can disengage bacterial clearance from inflammation and can thereby contribute to the persistence of microbial communities that drive periodontitis (Fig. 2). This, however, does not imply that all cases of periodontitis are initiated by *P. gingivalis*, which constitutes one of many factors that can contribute to the disruption of periodontal host-microbe homeostasis (see above).

## Anti-inflammatory interventions can control the periodontitis-associated microbiota

A clinical study that characterized the periodontal microbiota of progressing initial chronic periodontitis concluded that no species in baseline microbial samples alone were strongly associated with progressing periodontitis (Tanner *et al*., 2007). Interestingly, as an alternative interpretation, the authors suggested that periodontal inflammation may

determine the composition of the microbiota rather than vice-versa, in other words "the organisms are there *because* of the disease, which raises the possibility that periodontal pathogens may not predict future disease" (Tanner *et al*., 2007). The notion that inflammation fosters the growth of a pathogenic microbiota (Fig. 1) is consistent with experimental data showing that anti-inflammatory treatments diminish the periodontal bacterial burden. In a rabbit model of *P. gingivalis*-induced periodontitis, treatment with resolvin E1, a proresolving mediator of inflammation, not only resolved periodontal inflammation but also diminished the numbers of *P. gingivalis* and other Gram-negative periodontal bacteria (Hasturk *et al*., 2007). Similarly, treatment of *P. gingivalis*-induced periodontitis in mice with an antagonist of the complement C5a receptor (C5aR; CD88) reversed both inflammation and the outgrowth of the commensal microbiota caused by *P. gingivalis* colonization; *P. gingivalis* itself was essentially eradicated by the C5aR antagonist treatment (Abe *et al*., 2012; Hajishengallis *et al*., 2011). Moreover, treatment of mice undergoing ligature-induced periodontitis with meloxicam (a selective inhibitor of cyclooxygenase-2) caused a significant reduction of the periodontal bacterial load (Eskan *et al*., 2012). A recent study has identified a periodontal pathobiont (designated NI1060) that selectively accumulates in damaged periodontal tissue and thrives under inflammatory conditions, thereby becoming particularly pathogenic in causing bone loss (Jiao *et al*., 2013). More recently, topical treatment with a probiotic preparation (*Lactobacillus brevis* CD2) in the oral mucosa of mice undergoing ligature-induced periodontitis not only inhibited inflammation and bone loss but also exerted differential effects on the aerobic and anaerobic microbiotas (Maekawa & Hajishengallis, 2014). Specifically, the probiotic treatment with *L. brevis* CD2 resulted in significantly higher counts of aerobic bacteria and, conversely, significantly lower counts of anaerobic bacteria, as compared to the placebo-treated control group. This finding is consistent with the notion that periodontitis-associated bacteria are predominantly (if not exclusively) anaerobic and inflammophilic; therefore, their growth will be limited once inflammation is under control (tissue breakdown is minimal and hence the source of nutrients diminishes). However, an alternative (or additional) interpretation is that *L. brevis* CD2 might have a direct inhibitory effect on periodontal anaerobic bacteria and, conversely, a stimulatory effect on aerobic bacteria.

As discussed above, the capacity of *P. gingivalis* to colonize the murine periodontium and cause elevation of the total bacterial counts requires intact C5aR signaling (Abe *et al*., 2012; Hajishengallis *et al*., 2011; Maekawa *et al*., 2014b). *P. gingivalis* can activate C5aR by releasing the C5a fragment from complement component C5 through the action of its Arg-specific gingipains (Liang *et al*., 2011; Wang *et al*., 2010; Wingrove *et al*., 1992) (Fig. 2). Consistent with its ability to activate C5aR independently of the immunological activation of complement, *P. gingivalis* retains its capacity to colonize the periodontium of C3-deficient ($C3^{-/-}$) mice (Maekawa *et al*., 2014a), in which complement cannot be activated by immunological mechanisms (*i.e*., via the classic, lectin, or alternative pathway) (Ricklin *et al*., 2010). In $C3^{-/-}$ mice, *P. gingivalis* could additionally elevate the total microbiota counts, as determined one week post-inoculation. Intriguingly, however, six weeks post-inoculation, the total microbiota counts in *P. gingivalis*-colonized $C3^{-/-}$ mice were diminished relative to those of *P. gingivalis*-colonized WT mice and approached the microbiota counts of sham-inoculated $C3^{-/-}$ mice (Maekawa *et al*., 2014a). *P. gingivalis*-colonized $C3^{-/-}$ mice also

experienced significantly less periodontal inflammation and bone loss than *P. gingivalis*-colonized wild-type mice (Maekawa *et al*., 2014a). Therefore, although C3 is not required for *P. gingivalis* colonization and the initial elevation of the total microbiota counts, C3 is essential for the long-term sustenance of the dysbiotic microbiota and for maximal inflammatory bone loss. The reason why *P. gingivalis*-induced dysbiosis cannot be sustained in *C3*$^{-/-}$ mice is likely related to the diminished periodontal inflammation in these mice. This study also provides a model where the presence of a keystone pathogen such as *P. gingivalis* does not necessarily lead to disease in the absence of critical host signaling pathways. This could explain, at least in part, why the presence of *P. gingivalis* in the human periodontium is not always associated with disease (Abusleme *et al*., 2013; Haffajee *et al*., 1998); a possible scenario could be that hyporesponsive or lack-of-function polymorphisms attenuate inflammation and prevent disease development despite the presence of *P. gingivalis* or other organisms capable to act as keystone pathogens.

Similar conclusions about the role of inflammation in periodontal dysbiosis can be drawn from the investigation of aggressive forms of periodontitis, as in leukocyte adhesion deficiency. Mice deficient in the LFA-1 integrin (a model of leukocyte adhesion deficiency type I; LAD-I) experience overproduction of periodontal IL-17 and severe bone loss early in life, as do human LAD-I patients (Moutsopoulos *et al*., 2014). Interestingly, anti-IL-17 treatment not only inhibits inflammation and bone loss but also diminishes the periodontal bacterial burden (Moutsopoulos *et al*., 2014). Consistent with the animal model studies, a recent metagenomic study showed that the bacterial biomass of human periodontitis-associated biofilms increases with increasing periodontal inflammation (Abusleme *et al*., 2013).

These studies collectively suggest that the established pathogenic biofilm in periodontitis is inflammophilic. Apparently, inflammation generates an environment that is conducive for the selection and overgrowth of dysbiotic microbial species. Once such environment is established, the periodontitis-associated bacteria provoke further inflammation (to secure nutrients) which collaterally causes even more tissue damage (Fig. 1). Consistent with the notion that inflammation is exploited by the bacteria as a means that serves their nutritional needs, a study investigating the *in situ* community-wide transcriptome of the subgingival periodontitis-associated microbiota demonstrated increased expression of proteolysis-related genes and genes for iron acquisition and lipopolysaccharide synthesis (Duran-Pinedo *et al*., 2014).

## Therapeutic implications and conclusion

The above-discussed concepts could be summarized as follows. Nutrients derived from inflammatory tissue breakdown select for community members that are inflammophilic. Subsequently, the emerging community proactively induces inflammation to sustain itself. Bacteria-induced inflammation can fuel further changes to the biofilm and stabilize the transition to a disease-provoking microbiota. An obvious implication is that periodontitis can be treated by approaches distinct from conventional treatment, which involves mechanical removal or physical disruption of the tooth-associated biofilm. In this context, it should be noted that conventional periodontal treatment is often not sufficient by itself to

control destructive inflammation and many patients develop recurrent disease (Armitage, 2002; Colombo *et al*., 2012). From an ecological point of view, promising approaches could be those interfering with the environmental factors that drive the selection and enrichment of disease-causing bacteria. For instance, therapeutic interventions could be implemented aiming to alter the local environment by reducing the flow of the GCF or the site could be rendered less anaerobic through the use of oxygenating or redox agents (Marsh, 2003). The GCF flow could be readily reduced by anti-inflammatory approaches. Moreover, a recent study showed that the ratio of receptor activator of NF-κB ligand to osteoprotegerin (RANKL/OPG), which reflects osteoclastic activity, was not reduced following conventional periodontal treatment despite improved clinical parameters (Bostanci *et al*., 2011). This important finding implies that subclinical inflammatory events may be unaffected by standard modes of treatment, thus highlighting the need for adjunctive anti-inflammatory treatments. Several anti-inflammatory interventions have been tested in preclinical animal models of periodontitis, including non-human primates, which share key clinical, microbiological, and immunohistological features with the human disease (Assuma *et al*., 1998; Brecx *et al*., 1985; Holt *et al*., 1988; Kornman *et al*., 1981). Such efforts have targeted diverse inflammatory pathways, ranging from upstream events associated with inflammatory cell recruitment, to signaling pathways that amplify and propagate inflammation, as well as promoting the resolution of periodontal inflammation through the use of specific pro-resolution agonists (Abe *et al*., 2012; Assuma *et al*., 1998; Eskan *et al*., 2012; Graves, 2008; Hasturk *et al*., 2012; Kirkwood *et al*., 2007; Maekawa *et al*., 2014b). In conclusion, inflammation is not only the driving force for the destruction of periodontal tissues; from a microbial perspective, the importance of inflammation lies in its providing a source of nutrients that drive the selection and persistence of a disease-provoking microbiota. Taken together, it can be concluded that anti-inflammatory agents are central to the effective treatment of periodontitis as they are likely to control both dysbiosis and disease progression.

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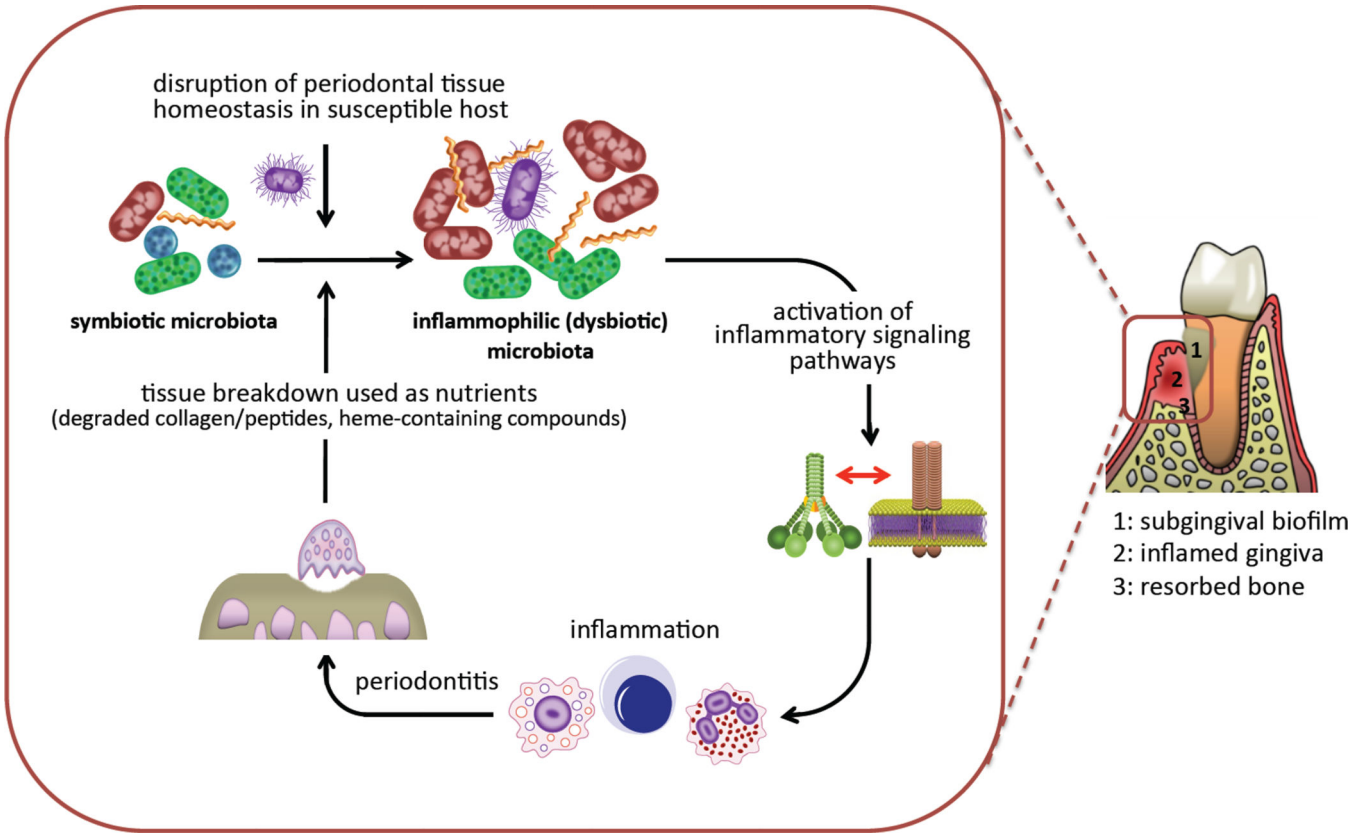


**Figure 1. Inflammation and dysbiosis in periodontitis**

Various factors including defects in the immuno-inflammatory status of the host and/or the presence of keystone pathogens can tip the balance from a symbiotic periodontal microbiota to a dysbiotic state. The inflammation caused by the dysbiotic microbiota depends in great part on crosstalk signaling between complement and pattern-recognition receptors and exerts two major and interrelated effects: it causes inflammatory destruction of periodontal tissue (including bone loss, the hallmark of periodontitis) which in turn provides tissue breakdown-derived nutrients for the bacteria. The latter further promotes dysbiosis and escalates tissue destruction, thereby generating a self-perpetuating pathogenic cycle. Therefore, the periodontitis-associated dysbiotic communities are ‘inflammo-*philic*’ (= loving or attracted to inflammation) in that they appear to have evolved to not only endure inflammation but also to take advantage of it for enhancing their adaptive fitness.

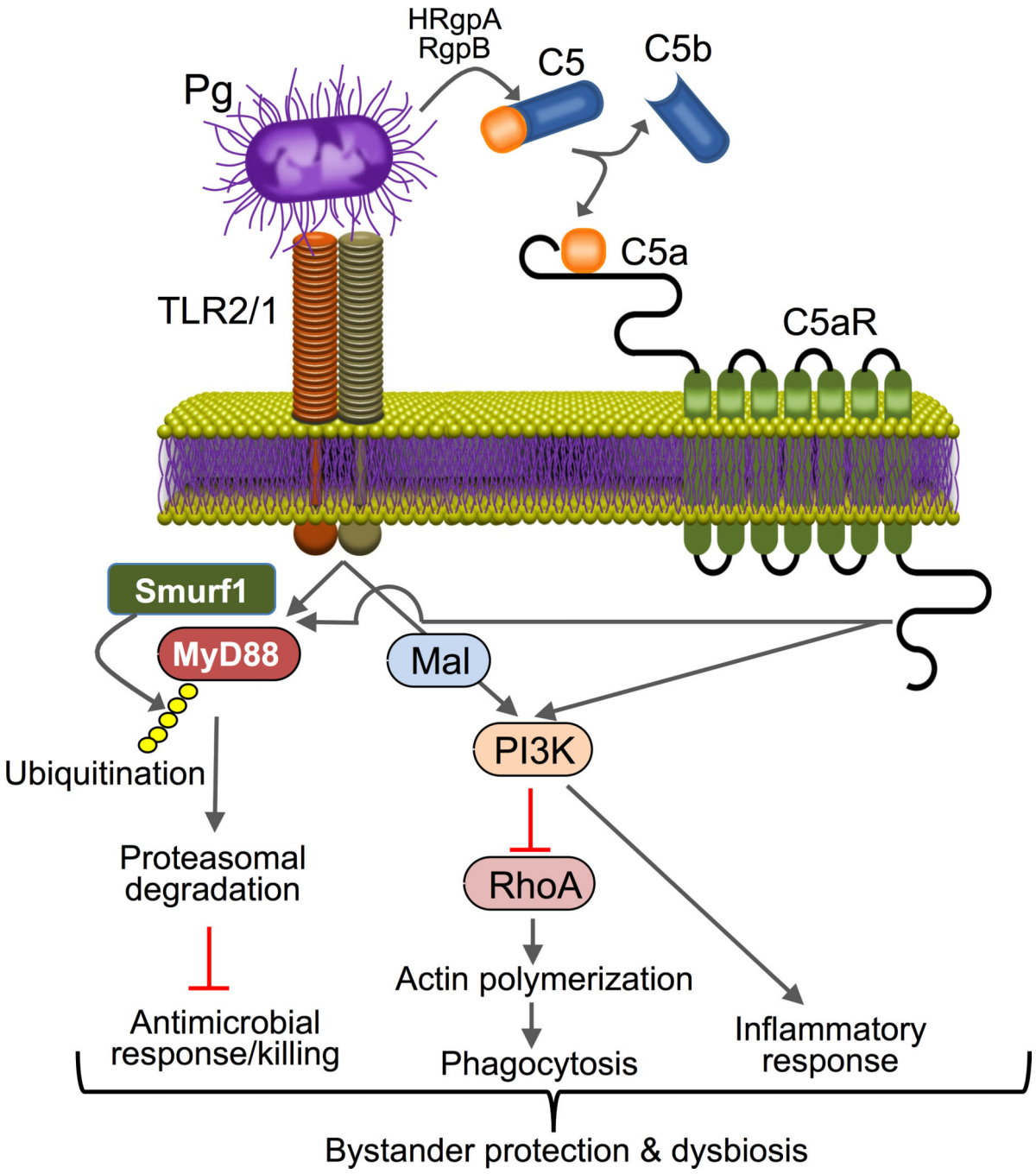


**Figure 2. Model of *P. gingivalis* subversion of neutrophils leading to dysbiotic inflammation**

*P. gingivalis* co-activates TLR2 and C5aR in neutrophils and the resulting crosstalk leads to E3 ubiquitin ligase Smurf1-dependent ubiquitination and proteasomal degradation of MyD88, thereby inhibiting a host-protective antimicrobial response. Moreover, the C5aR-TLR2 crosstalk activates PI3K, which prevents phagocytosis through inhibition of RhoA activation and actin polymerization, while stimulating an inflammatory response. In contrast to MyD88, Mal is a component of the subversive pathway acting upstream of PI3K. The integrated mechanism provides 'bystander' protection to otherwise susceptible bacterial

species and promotes polymicrobial dysbiotic inflammation *in vivo*. From Maekawa *et al*., 2014b with permission.