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Role of complement in host-microbe homeostasis of the periodontium

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Abstract

Complement plays a key role in immunity and inflammation through direct effects on immune cells or via crosstalk and regulation of other host signaling pathways. Deregulation of these finely balanced complement activities can link infection to inflammatory tissue damage. Periodontitis is a polymicrobial community-induced chronic inflammatory disease that can destroy the tooth-supporting tissues. In this review, we summarize and discuss evidence that complement is involved in the dysbiotic transformation of the periodontal microbiota and in the inflammatory process that leads to the destruction of periodontal bone. Recent insights into the mechanisms of complement involvement in periodontitis have additionally provided likely targets for therapeutic intervention against this oral disease.

Keywords

Complement; C5a receptor; periodontitis; dysbiosis; *P. gingivalis*

1. Introduction

Complement can be produced locally or systemically [1] and plays important roles in immunity and inflammation through direct effects on innate and adaptive immune cells or through crosstalk and regulation of other signaling pathways [2,3]. Complement activities are therefore not restricted to a linear cascade of events but involve a network of interactions with other systems to better coordinate the host response to infection or other insults. These connections of complement can enhance innate immune defense through synergy with Toll-like receptors (TLRs) [3], provide a barrier against the spread of invading bacteria by potentiating local clotting [4], and replenish the immune system through mobilization of

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hematopoietic stem/progenitor cells from the bone marrow [5,6]. Complement also influences the activation and differentiation of T-cell subsets [7,8].

Besides the classical group of serum proteins (C1-9), the integrated complement system includes pattern-recognition molecules, convertases and other proteases, regulators, and receptors for interactions with immune mediators [2]. The complement cascade can be triggered via distinct pathways (classical, lectin, or alternative), which converge at the third complement component (C3). The activation of the classical pathway is initiated by antigen-antibody complexes recognized by the C1q subunit of C1, whereas the lectin pathway can be triggered through interaction of a secreted pattern-recognition molecule (the mannose-binding lectin; MBL) with specific carbohydrate groups on microbial surfaces. Both the classical and lectin pathways proceed through C4 and C2 cleavage to generate the classical/lectin C3 convertase. The alternative pathway is initiated by low-level, spontaneous hydrolysis of C3 to C3[H_2O], which forms the initial alternative pathway C3 convertase in the presence of factors B (fB) and D (fD). As long as there is no sufficient negative regulation (as is normally the case of microbes or other non-self surfaces), this initiation is followed by rapid propagation of the alternative pathway through an amplification loop [2,7]. The alternative pathway can also be triggered by bacterial lipopolysaccharide and lipooligosacharide via the plasma protein properdin attached to bacterial surfaces [9,10] and can potentially contribute to ≥80% of the total complement activation [11]. C3 activation by pathway-specific C3 convertases leads to the generation of effector molecules involved in (a) the recruitment and activation of inflammatory cells (*e.g.*, the C3a and C5a anaphylatoxins that activate specific G-protein-coupled receptors, C3aR and C5aR [CD88], respectively); (b) microbial opsonization and phagocytosis (*e.g.*, through the C3b opsonin); and (c) direct lysis of targeted pathogens (by means of the C5b-9 membrane attack complex [MAC])[2].

As alluded to above, complement is not normally activated on the surface of host cells and tissues. However, disruption of the regulatory mechanisms involved can lead to excessive complement activation, inflammation, and damage to host tissues. For instance, deficiencies, hypo-functional polymorphisms, or mutations in complement regulators have been implicated in the development of local or systemic diseases, such as age-related macular degeneration and systemic lupus erythematosus [2,12,13]. Microbial pathogens may also contribute to complement deregulation or dysfunction. In this regard, pathogens can hijack negative regulators of complement to protect themselves against host defense mechanisms, or they can degrade regulatory molecules that protect host tissues or cells [14–16]. Moreover, pathogens can exploit complement receptors to proactively promote their survival and persistence in the host [17]. These mechanisms can contribute to defective bacterial clearance and enhanced inflammation, thereby promoting infection-driven inflammatory diseases.

Periodontitis is an inflammatory disease in which complement appears to form a major link between infection and inflammation [18]. Chronic periodontal inflammation is initiated and perpetuated by a dysbiotic microbiota and may lead to tooth loss as a result of the destruction of the supporting alveolar bone [19,20]. This is a highly prevalent disease, affecting nearly half of U.S. adults [21]. In its most severe form, which affects 8.5% of U.S. adults [21], periodontitis can influence systemic health and increase the risk for atherosclerosis, diabetes, and possibly rheumatoid arthritis [22–25].

In this review, we summarize and discuss the published evidence supporting a crucial role for complement in the initiation and progression of periodontitis (Table 1). Specifically, excessive activation of complement or subversion of its normal functions contributes to the breakdown of host-microbe homeostasis in the periodontal tissue (periodontium), thereby

precipitating inflammatory disease. This evidence comes from both clinical observations and experimental animal studies.

2. Role of the microbiota in periodontitis

In order to better understand the interplay between bacteria and complement in periodontitis, it is instructive to first consider the role of bacteria in the pathogenesis of this chronic oral inflammatory disease. Until fairly recently, the identities of the organisms associated with periodontal disease or health were restricted to those that could be cultured in the laboratory. Cultural characterization of the periodontal microbiota in the late 1970s and early 1980s revealed dramatic compositional changes in disease as compared to health [26–31]. One way to interpret these findings was that the disease-associated microbiota contained novel pathogenic species that were either absent or barely detectable in health. On the basis of cluster analysis and association with different disease severities, oral bacteria were historically divided into six groups of different potential pathogenicity, commonly referred to by their color-coded designations [32]. Foremost among these groups was the so-called "red complex" a group of three, Gram-negative anaerobic species, including *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*, the detection of which was strongly associated with each other and with diseased sites [32]. Also associated with periodontal lesions, the "orange" complex comprised species members of the genus *Prevotella*, *Fusobacterium,* and *Campylobacter,* as well as *Streptococcus constellatus* and *Eubacterium nodatum*. The other four complexes ("blue", "yellow", "green", and "purple") primarily consisted of early colonizers of the tooth surfaces [32].

With the advent of culture-independent, molecular-based methods of bacterial identification and enumeration, such as 16S rDNA amplification and high-throughput sequencing, our understanding of the bacterial composition of the periodontal region has changed [33–35]. The in-depth study of thousands of plaque samples derived from a variety of clinical periodontal conditions has demonstrated a more heterogeneous and diverse periodontal microbiota than previously thought [33–35]. In addition to the consensus periodontal pathogens, *P. gingivalis*, *T. forsythia,* and *T. denticola*, newly recognized non- or poorly cultivable organisms that increase in number in diseased sites include the Gram-positive *Filifactor alocis* and species in the genera *Prevotella, Megasphaera, Selenomonas,* and *Desulfobulbus* [33,34,36–39]. Many of these newly recognized organisms show as good a correlation (or better) with disease as does the classical red complex [37,39,40]. It is now increasingly recognized that chronic periodontitis is not a bacterial infection in the classical sense, *i.e.*, caused by a single or a limited number of pathogens. Rather, periodontal disease is the result of a polymicrobial community-induced perturbation of host homeostasis in susceptible individuals [20,41]. Bacterial constituents of these communities often exhibit synergistic interactions that can enhance colonization, persistence, or virulence, and some bacteria may be involved in the breakdown of periodontal homeostasis, whereas other may trigger destructive inflammation once homeostasis is disrupted [20].

3. Mechanisms used by periodontal bacteria for protection against complement

The space between the free gingiva and the tooth surfaces, known as the gingival crevice, constitutes a niche for periodontitis-associated microbial communities [41]. The gingival crevice is bathed with an inflammatory exudate, termed gingival crevicular fluid (GCF) [42]. In a clinically healthy periodontium, in which the tooth-associated biofilm is usually confined to the gingival margin, the GCF represents a slow-flowing transudate of plasma proteins. However, if the biofilm is left undisturbed for 2–4 days, the biofilm enters the crevice by proliferation and spreading or by relocation of dislodged bacteria. The host

response is therefore escalated and manifested by increased flow of GCF (in part due to increased vascular permeability of the subepithelial blood vessels) and chemotactic recruitment of inflammatory cells, mostly neutrophils [43]. Under inflammatory conditions, the GCF contains complement at up to 70% to 80% of its concentration in serum, although certain components can be found at higher levels in GCF reflecting local production of complement [44–47]. Therefore, the periodontal bacteria constantly encounter complement and, in cases of chronic periodontitis, it is reasonable to think that the bacteria have evolved mechanisms to resist its antimicrobial actions.

Studies with several periodontal bacteria, such as *P. gingivalis*, *Prevotella intermidia*, *T. forsythia*, and *T. denticola*, indicate that they interact with complement in complex ways that include both inhibitory and stimulatory effects [15,16,48,49]. This seemingly contradictory microbial behavior is probably due to the dynamics of survival tactics of periodontal bacteria: on the one hand trying to evade immune clearance, and on the other to stimulate inflammation and the flow of GCF as a source of nutrients [16] (Fig. 1).

P. gingivalis expresses Arg- and Lys-specific cysteine proteinases known as gingipains, which can degrade C3, thereby potentially inhibiting complement activation regardless of the initiation pathway involved [44]. All three gingipains can degrade C3, although the Arg-specific enzymes (HRgpA and RgpB) are more potent in this regard than the Lys-specific gingipain (Kgp) [44]. As a consequence, the deposition of opsonins or the C5b-9 MAC on the pathogen surface is suppressed, unless the activity of the gingipains is inhibited by chemical or genetic means [50,51]. Consistent with these findings, *P. gingivalis* displays exquisite resistance to the lytic action of complement in vitro [44,50]. Unexpectedly, however, Arg- and Lys-gingipain deletion mutants maintained resistance to killing in 20% normal human serum (a similar viability to that seen in heat-inactivated serum) despite increased deposition of complement fragments or complexes (C3d and C5b-9 MAC) [50]. This finding suggested that the mechanism of serum resistance is largely independent of the Arg- and Lys-gingipains and has, in fact been attributed to the presence of a surface anionic polysaccharide (APS; now known as A-LPS, *i.e.*, LPS with APS repeating units [52]), the lack of which renders *P. gingivalis* extremely sensitive to complement killing in 20% normal serum [50]. Taken together, these data raise the following question: What is the biological significance of gingipain-dependent C3 degradation if *P. gingivalis* is inherently resistant to the lytic action of complement? Since the inhibitory mechanisms of *P. gingivalis* against complement activation are “leaky” [44], it is possible that the A-LPS-dependent resistance represents a reinforcing mechanism to provide maximal protection against complement. In addition, or alternatively, *P. gingivalis* may have evolved the capacity to inactivate complement to protect susceptible species occupying the same subgingival niche (this could be one of the mechanisms for the keystone pathogen status of *P. gingivalis*; see below). In this regard, incubation of human serum with purified gingipains or whole cells of *P. gingivalis* has been shown to cause a drastic decrease in the bactericidal activity of the serum against susceptible organisms [44].

The complex nature of *P. gingivalis*-complement interactions is also suggested by the observation that all three gingipains (HRgpA, RgpB, and Kgp) can activate the C1 complex in serum at relatively low concentrations, resulting in the deposition of C1q on inert surfaces or on the bacteria themselves [44]. It is reasonable to speculate that *P. gingivalis* activates complement when present in low numbers, while attempting to establish infection. This complement activation is unlikely to eliminate *P. gingivalis* (since it is inherently resistant to complement-mediated lysis), but the resulting local inflammatory response may provide *P. gingivalis* with precious nutrients, such as GCF-derived hemin, a source of essential iron. At later stages of infection, when the concentration of proteases is high enough to destroy C3

and inhibit complement activation, *P. gingivalis* may promote the survival of the entire biofilm community by helping bystander bacteria evade complement killing.

Certain strains of *Prevotella intermedia* express and secrete interpain A (InpA), a streptopain-like cysteine protease, which can also degrade C3 and contribute to resistance against the antibacterial activity of complement [53]. Interestingly, *P. intermedia* shares an important feature with *P. gingivalis*. At low concentrations, interpain, like the gingipains, is able to activate the C1 complex in serum, causing deposition of C1q on bacterial surfaces [53]. Moreover, the interpain of *P. intermedia* acts in synergy with the gingipains of *P. gingivalis* in inactivating complement in vitro [53]. Such synergy may be of relevance for the in vivo pathogenicity of mixed biofilms, given that the two organisms can co-aggregate [54,55]. Such synergy may occur even if the two species do not intimately interact in subgingival biofilms. Since the gingipains and the interpain are secreted proteases, they can diffuse, reach, and protect bystander bacterial species, which would otherwise be eliminated by the complement bactericidal activity.

T. forsythia, another important species associated with periodontitis [56], also possess mechanisms for escaping complement. *T. forsythia* expresses karilysin, a metalloproteinase that mediates resistance to killing by human complement by acting at different stages of the complement cascade: Specifically, karilysin inhibits the classical and lectin pathways by degrading MBL, ficolin-2, ficolin-3, and C4, whereas it blocks the terminal pathway by degrading C5 [57]. Karilysin synergizes with interpain and gingipains in these subversive functions, suggesting that *P. gingivalis*, *P. intermedia,* and *T. forsythia* together can better protect susceptible bystander bacterial species and promote the survival of the entire biofilm [57].

In addition to the degradation of key complement components, other evasion mechanisms depend upon the ability of pathogens to hijack and employ circulating complement regulators [14,49]. In this regard, *P. gingivalis* uses its HRgpA to capture the circulating C4b-binding protein (C4BP) on the bacterial cell surface, thereby acquiring the ability to negatively regulate the classical pathway C3 convertase [58]. *Aggregatibacter actinomycetemcomitans* uses its outer membrane protein 100 to bind the alternative pathway inhibitor factor H and acquire resistance to complement killing in serum [59]. *P. intermedia* binds the serine protease factor I (FI), a major inhibitor of complement that degrades C3b and C4b in the presence of cofactors such as C4BP and factor H [60]. Importantly, *P. intermedia* also binds the cofactors factor H and C4BP leading, respectively, to increased degradation of C4b and C3b [60].

T. denticola expresses a 11.4-kDa cell surface lipoprotein that can bind factor H [61]. Strikingly, however, once full-length factor H becomes associated with *T. denticola*, the organism uses its serine protease dentilisin to generate a 50-kDa factor H fragment that remains attached to the bacterial surface [61]. This seems paradoxical, since dentilisin appears to counteract the action of the factor H-binding protein. An interesting question therefore is whether the attached fragment retains useful complement inhibitory activity to protect *T. denticola* against complement. Alternatively, if dentilisin does inactivate factor H, this protease may deregulate complement and promote local inflammation.

The *T. denticola* dentilisin can also hydrolyze the α-chain of C3 and generate iC3b [62], consistent with an early study demonstrating opsonization of *T. denticola* with iC3b [63]. Intriguingly, iC3b-mediated phagocytosis is often associated with weak killing mechanisms or even immunosuppressive signaling [64–67] and may be exploited by certain pathogens. Indeed, *P. gingivalis*, *Mycobacterium tuberculosis*, *Bordetella pertussis,* and HIV-1 promote

their intracellular survival by exploiting complement receptor-3-mediated entry, either by direct interaction with the receptor or upon opsonization with iC3b [14,17,68–70].

4. Periodontal bacteria proactively utilize complement to cause inflammation

As alluded to earlier, periodontal bacteria can proactively activate complement using virulence factors (gingipains or interpain at relatively low concentrations) that directly interact with the C1 complex. Although high concentrations of *P. gingivalis* gingipains or *P. intermedia* interpain inhibit the complement cascade, the interpain can directly release anaphylatoxin C3a, while gingipains can release C5a [44,53,71]. Both enzymes preferentially attack the α-chains of C3 and C5 [44,53,72]. Since the anaphylatoxins are potent mediators of inflammatory responses [2,73], their local generation at sites heavily populated with bacteria can contribute to the inflammatory destruction of the periodontium and the generation of tissue breakdown nutrients for the bacteria (Fig. 1). Mechanistically, local complement activation can promote periodontal inflammation through C5a-induced vasodilation, increased vascular permeability, the flow of inflammatory exudate, and chemotactic recruitment of inflammatory cells, especially neutrophils [16,74]. Moreover, C5a can be also exploited by the bacteria for the immune subversion of leukocytes (see below).

Membrane-anchored negative regulators of complement, such as CD46, CD55, and CD59, protect host cells from unwarranted complement attack [2,12]. In interacting with oral epithelial cells, *P. gingivalis* causes the shedding of membrane-anchored CD46, which as a soluble protein can lead to IL-8 secretion from the epithelial cells or become degraded by the Kgp gingipain of the bacterium [75]. Regardless of whether shed CD46 can further promote inflammatory responses, its removal from the epithelial cell surface can render a cell susceptible to the destructive effects of complement activation. It should be noted, however, that under inflammatory conditions, oral epithelial cells upregulate the expression of CD46, CD55, and CD59 [76], presumably to prevent cell lysis by excessive complement activation and/or replenish the loss of these membrane-bound regulators as a result of attack by bacterial proteases.

Although CD46 was originally appreciated for its role in preventing complement deposition on host cells and tissues, recent work has ascribed additional regulatory functions to CD46, including the ability to regulate T-helper type 1 immune responses [77]. This may explain why a number of microbial pathogens exploit CD46 to promote their fitness [78]. *Fusobacterium nucleatum*, a periodontal bacterium that forms a co-aggregating bridge between early and late colonizers of the dental biofilm and may occur in deep periodontal pockets along with *P. gingivalis* [79,80], has been shown to bind CD46 on oral epithelial cells [76]. This interaction upregulates IL-6, IL-8, and matrix metalloproteinase (MMP)-9 at the mRNA and protein level and has been interpreted as a mechanism that can contribute to host tissue destruction in periodontitis [76].

Taken together, these data suggest that periodontal bacteria have evolved mechanisms to proactively regulate inflammation to their own advantage, since host tissue destruction can provide them with nutrients and new niches (as a result of deeper periodontal pockets) (Fig. 1). However, this inflammatory destruction of host tissue could be a genuine advantage only if, at the same time, the periodontal bacteria have evolved to endure inflammation. This concept is discussed below.

5. Exploitation of complement as a mechanism to cause dysbiosis and periodontitis

As discussed above, *P. gingivalis* proactively generates C5a. All three gingipains (especially HRgpA and RgpB) act in a C5 convertase-like manner and generate biologically active C5a through limited degradation of C5 [44,72]. When C5 is oxidized by hydroxyl radicals (as may occur in a local inflammatory environment with neutrophil-released oxidants), the gingipains generate elevated C5a activity [81]. Furthermore, *P. gingivalis* HRgpA and RgpB activate prothrombin to form thrombin [82] which, in turn, generates biologically active C5a by acting as a C5 convertase [83]. C5a can also be generated in vivo by *P. gingivalis,* but not by an isogenic mutant lacking all three gingipain genes [84].

C5a can potentially play a key role in host defense against infection [85]. However, not only is *P. gingivalis* survival not impaired by C5a, but it is enhanced. Mechanistically, *P. gingivalis*-generated C5a activates C5aR and stimulates Gαi-dependent intracellular Ca^{2+} signaling, which synergistically enhances an otherwise weak cAMP response by *P. gingivalis*-induced TLR2 activation alone [71]. The resulting crosstalk sustains high production of cAMP and leads to the activation of the cAMP-dependent protein kinase A, which inactivates the glycogen synthase kinase-3β. This, in turn, impairs the nitric oxide-dependent killing of *P. gingivalis* [71]. This C5aR-TLR2 subversive crosstalk undermines the killing function of macrophages, although it is yet to be shown whether a similar mechanism protects *P. gingivalis* against neutrophils.

The *P. gingivalis*-induced C5aR-TLR2 crosstalk also inhibits TLR2-induced IL-12p70, but it upregulates inflammatory and bone-resorptive cytokines such as IL-1β, IL-6, IL-17, and TNF [84,86]. This inhibition enables *P. gingivalis* to escape IL-12p70-dependent immune clearance in vivo and to cause inflammatory bone loss in a mouse model of periodontitis [84,86]. Because the C5aR-TLR2 crosstalk inhibits only a subset of TLR2 signaling events, C5aR is considered to be a “modulatory” receptor for TLRs, as opposed to “TLR inhibitory receptors” such as IL-10 receptor or TGF-β receptor, which inhibit most, if not all, inflammatory responses [87]. Therefore, by generating C5a to locally regulate C5aR, *P. gingivalis* can not only promote its survival but also contribute to destructive inflammation. The induction of totally immunosuppressive signaling would not be a safe option for *P. gingivalis* because *P. gingivalis* is a strictly asaccharolytic organism, and its survival and growth depends crucially on inflammatory tissue breakdown products (degraded proteins, as well as hemin for essential iron) [16].

Intriguingly, the manipulation of the periodontal C5aR response by *P. gingivalis* benefits the entire microbial community, which becomes dysbiotic (altered composition and increased total counts) and causes inflammatory periodontal bone loss [88] (Fig. 2). Strikingly, *P. gingivalis* causes these community-wide effects while present at low colonization levels (<0.01% of the total bacterial counts) in the periodontal tissue. Consistent with a C5aR requirement for disease pathogenesis, *P. gingivalis* fails to alter the microbiota in C5aR-deficient mice, which are also protected against periodontal inflammation and bone loss [88]. These data suggest that dysbiosis is required for inflammatory bone loss. Furthermore, the essential participation of the periodontal microbiota at large in the disease pathogenesis was confirmed by the inability of *P. gingivalis* to cause bone loss by itself, *i.e.*, in germ-free mice, despite colonizing this host [88].

Mice deficient in TLR2 are also resistant to inflammatory periodontal bone loss [84,89]. The fact that mice deficient either in C5aR or TLR2 are highly and similarly resistant to periodontitis is consistent with the notion that a crosstalk between the two receptors is synergistically involved in the disease process (indeed, as discussed above, C5aR-TLR2

crosstalk acts in synergy with inflammation and mediates immune subversion by *P. gingivalis* [71,84,86]). This in turn suggests that blockade of just one of the two receptors may be sufficient to inhibit the development of periodontitis. Indeed, local intragingival treatment of mice with a C5aR antagonist reverses *P. gingivalis*-induced dysbiosis [88] and prevents or halts the progression of periodontitis, depending on whether the antagonist is given in a preventive or therapeutic setting [86].

The ability of *P. gingivalis* to orchestrate inflammatory disease through community-wide effects, while being a quantitatively minor constituent of the periodontal microbiota, has prompted its characterization as a keystone pathogen, in analogy to the crucial role of a keystone that holds an entire arch together [88,90,91]. It is also plausible that keystone pathogens underlie the pathogenesis of several other polymicrobial inflammatory diseases (*e.g.*, inflammatory bowel disease), a concept that would lead to a better understanding of their etiology and to targeted diagnostic and treatment modalities [92].

Although 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 [93]. In non-human primates, in which *P. gingivalis* is a natural inhabitant of the periodontal biofilm, a gingipain-based vaccine causes a reduction both in Pg numbers and the total subgingival bacterial load [94], suggesting that the presence of *P. gingivalis* benefits the entire biofilm. The keystone pathogen concept is also consistent with *P. gingivalis* being a quantitatively minor constituent of human periodontitis-associated biofilms [26,40,95], despite its high prevalence and association with progressive bone loss in periodontal patients [96,97]. Importantly, the mouse model is similar to the situation in humans in terms of innate defense status, including complement and neutrophil transit [98], and, moreover, a significant increase in the total microbial load is also observed in human periodontitis as compared to health [99].

6. Potential for complement-targeted therapeutics in periodontitis

The data obtained from animal models suggest that complement inhibition may have therapeutic potential in human periodontitis, where complement involvement is supported by clinical and histological observations (Table 1). Complement components and cleavage products covering the whole complement cascade have been detected in chronically inflamed gingiva, although they are undetected or at lower levels in healthy control samples [45,47,100–104]. The gingival crevicular fluid (GCF) from periodontitis patients displays complement-dependent hemolytic activity, suggesting the presence of a functional complement system (C1–C9) in gingival inflammatory exudates [100,104]. Moreover, the GCF of patients contains activated complement fragments at higher concentrations than in the GCF from healthy individuals [46,105–108]. Importantly, induction of experimental gingival inflammation in human volunteers causes a progressive elevation of complement cleavage products that is correlated with increased clinical indices of inflammation [108]. C3 is among the top 5% of genes that are most strongly downregulated following periodontal therapy [109]. Moreover, C3 conversion to C3c in GCF decreases dramatically after periodontal therapy [110]. Interestingly, a single nucleotide polymorphism of C5 (rs17611), which is associated with increased serum C5 levels and susceptibility to liver fibrosis (a complement-associated disease) [111], has been shown to be more prevalent in periodontitis patients than in healthy controls [112]. An immunohistochemical study showed weaker expression of CD59 in the gingiva of periodontitis patients than in healthy controls, suggesting reduced protection of diseased tissues against possible tissue damage by autologous MAC formation [103].

The involvement of complement in human periodontitis has recently prompted intervention studies in preclinical models, on the basis of mechanistic studies implicating C5aR in disease pathogenesis. When used preventively, local intragingival injection of a C5aR antagonist (C5aRA; PMX-53) in mice completely inhibits *P. gingivalis*-induced bone loss [86]. Mice receiving this treatment elicit minimal inflammatory responses (TNF, IL-1β, and IL-17), like those of sham-infected mice. C5aRA is able to protect against inflammatory bone loss even when it is administered 2 weeks after the onset of experimental periodontitis [86]. This is important, since periodontal treatment is more likely to be implemented in a therapeutic than a preventive mode. Nevertheless, preventive intervention may be appropriate for individuals at high risk for periodontitis, such as cigarette smokers, diabetic patients, or individuals with systemic diseases affecting neutrophil function [113].

Since periodontitis fundamentally represents a disruption of host-microbe homeostasis [20,41] and is associated with multiple etiologies and disease modifiers [19,25,114,115], *P. gingivalis* is not likely to be involved in all cases of periodontitis. C5aRA is able to inhibit periodontal bone loss in a mouse model in which periodontitis is induced independently of *P. gingivalis*, specifically the “ligature-induced periodontitis model,” In this model, a silk ligature is placed around the molar teeth, resulting in massive local bacterial accumulation and rapid induction of severe bone loss in specific-pathogen-free (but not germ-free) animals [116]. Mice locally treated with C5aRA at the ligated sites display significantly decreased gingival inflammation and about 50% less bone loss when compared to controls [86]. In a ligature-induced periodontitis study in rats, a PMX53 analog, PMX205, was administered in the drinking water prior to and during the disease, and it inhibited bone loss by <20% when compared to controls [117]. These differences in efficacy might be due to the different modes of administration used, although the different animal species used might have been another contributory factor. Modes of local administration, which restrict the action of C5aRA to the periodontal tissue, are likely to be safer, since systemic inhibition of complement may predispose to increased susceptibility to microbial infections.

7. Conclusions

In summary, complement is a target of immune subversion that leads to the dysbiotic transformation of the microbiota, which in turn causes complement-dependent destructive inflammation (Fig. 1). Specifically, *P. gingivalis* exploits C5aR to impair antimicrobial (but not proinflammatory) leukocyte responses, leading to uncontrolled growth and altered composition of the periodontal microbiota at large, which precipitates periodontitis [71,88,91,92] (Fig. 2). A better understanding of the role of the various complement pathways in periodontitis may identify additional targets for additional treatment options. For instance, blocking complement activity upstream of C5aR may be more beneficial if additional pathways contribute to the disease. This notion is supported by unpublished studies from our group: In ligature-induced periodontitis studies, C3-deficient mice or non-human primates locally treated with the C3 inhibitor compstatin [123–126] are protected against periodontal inflammation and bone loss.

The high prevalence of periodontitis (>47% of US adults, with 8.5% presenting with severe periodontitis) [21], its economic burden [118,119], and the fact that many periodontitis cases are refractory to conventional therapy [120,121] underscore the importance of implementing innovative and cost-effective therapeutic interventions. Although these efforts currently involve preclinical models, the availability of complement-specific drugs that have already undergone successful safety trials for other diseases [122–126] indicates a potential for rapid translation to the clinic.

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Highlights

- Human periodontitis is associated with increased complement activation
- Periodontal bacteria evade and subvert complement
- Experimental periodontitis is mediated by complement-dependent dysbiosis
- Antagonistic blockade of C5a receptor protects against experimental periodontitis

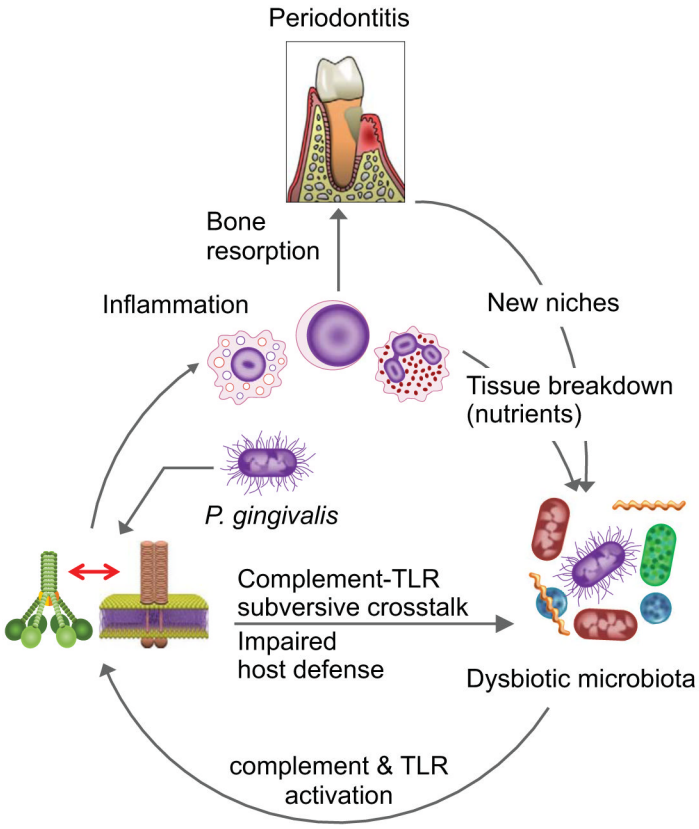

Fig. 1. Subversion of host immuno-inflammatory surveillance and destructive inflammation in periodontitis

Colonization by *P. gingivalis* impairs innate immunity by subverting complement-TLR crosstalk, leading to increased numbers of periodontal bacteria and therefore enhanced inflammation through the activation of synergistic complement and TLR pathways. The inflammatory environment is favorable to further bacterial growth, since the gingival inflammatory exudate is a rich source of nutrients (*e.g.*, degraded host proteins and hemin, a source of essential iron). These environmental changes, moreover, can alter the composition of the oral microbiota, favoring those bacteria (*e.g.*, proteolytic and asaccharolytic organisms) that can better exploit these environmental changes. These changes result in even higher inflammation and bone resorption, leading to increased niche space (deeper periodontal pockets) for the bacteria, thereby perpetuating a vicious cycle of periodontal tissue destruction. Adapted from Hajishengallis et al (ref. 92).

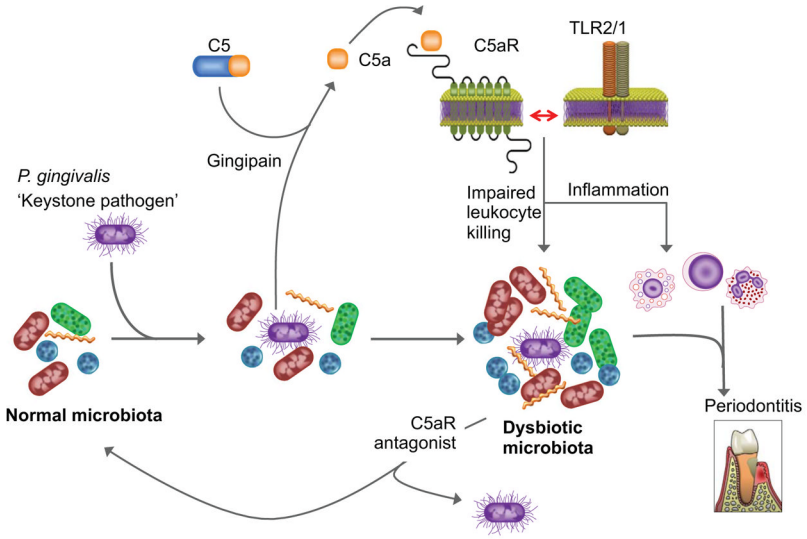

Fig. 2. *P. gingivalis*-induced dysbiosis in periodontitis

P. gingivalis exploits C5aR signaling and inhibits the host defense, leading to an altered composition and increased numbers of the periodontal microbiota which, in turn, cause complement-dependent periodontal inflammation and bone loss. This community-wide effect can occur at low colonization levels of *P. gingivalis* and requires its Arg-specific gingipain activity, acting in a C5 convertase-like manner and thus cleaving C5 and generating high levels of C5a locally. C5a-induced activation of C5aR triggers inflammation and is also crucially involved in subversive crosstalk with the TLR2/1 complex that impairs macrophage killing. The ability of *P. gingivalis* to orchestrate inflammatory disease by remodeling a symbiotic microbiota into a dysbiotic state, while being a minor constituent of this community, qualifies it as a keystone pathogen. This pathologic process is reversible, since C5aR blockade promotes the clearance of *P. gingivalis* and reverses its dysbiotic effects. Adapted from Hajishengallis et al (ref. 92).

Table 1

Clinical and experimental evidence for complement involvement in periodontitis

Observations	**Refs.**
Complement components and cleavage products covering the whole complement cascade are detected in chronically inflamed gingiva and in GCF of patients; undetected or at lower levels in healthy control samples.	[45–47, 100– 105]
Induction of experimental gingival inflammation in humans causes progressive elevation of complement cleavage fragments correlating with increased clinical inflammatory parameters.	[108]
C3 conversion to C3c in GCF increases with increasing periodontal pocket depth but decreases dramatically after periodontal therapy	[110, 127]
Possible reduced expression of complement negative regulators in periodontitis: (a) Reduced expression of CD59 in the gingiva of periodontitis patients compared to healthy controls; (b) *P. gingivalis* causes shedding and degradation of membrane-anchored CD46.	[75, 103]
Single nucleotide polymorphism of C5 (rs17611) significantly more prevalent in periodontitis patients than in healthy controls.	[112]
Periodontal bacteria (*e.g.*, *P. gingivalis*, *T. forsythia*, *T. denticola*, *P. intermedia. A. actinomycetemcomitans*) possess mechanisms for complement manipulation; evasion of antimicrobial effects and promotion of inflammatory responses.	[44, 50, 51, 53, 57–61, 71, 84, 128].
C5aR-deficient mice are resistant to dysbiosis and periodontitis.	[84, 88]
C5aR antagonist reverses dysbiosis and prevents or halts the progression of mouse periodontitis.	[86, 88]