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Complement inhibition in pre-clinical models of periodontitis and prospects for clinical application

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Abstract

Periodontitis is a dysbiotic inflammatory disease leading to the destruction of the tooth-supporting tissues. Current therapies are not always effective and this prevalent oral disease continues to be a significant health and economic burden. Early clinical studies have associated periodontitis with elevated complement activity. Consistently, subsequent genetic and pharmacological studies in rodents have implicated the central complement component C3 and downstream signaling pathways in periodontal host-microbe interactions that promote dysbiosis and inflammatory bone loss. This review discusses these mechanistic advances and moreover focuses on the compstatin family of C3 inhibitors as a novel approach to treat periodontitis. In this regard, local application of the current lead analog Cp40 was recently shown to block both inducible and naturally occurring periodontitis in non-human primates. These promising results from non-human primate studies and the parallel development of Cp40 for clinical use highlight the feasibility for developing an adjunctive, C3-targeted therapy for human periodontitis.

Keywords

complement; C3; therapeutics; compstatin Cp40; primate models; inflammation; periodontitis

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1. Introduction

Periodontitis is an oral disease driven by dysregulated inflammation induced by polymicrobial dysbiotic communities that form on subgingival tooth sites [1]. The disease can lead to the destruction of the periodontium (*i.e.*, the tooth-supporting structures, including gingiva, periodontal ligament, and the alveolar bone) and, if untreated, can lead to tooth loss and possibly impaired mastication [2]. Nearly half of adults in the U.S. are affected by some form of periodontal disease [3]. In its severe form that afflicts nearly 10% of adults [3, 4], periodontitis is associated with increased risk of certain systemic conditions, such as atherosclerosis and rheumatoid arthritis [5, 6].

As alluded to above, periodontitis is not a bacterial infection in the classical sense, *i.e.*, it is not caused by specific exogenous pathogen(s) [7, 8]. Moreover, the implicated resident dysbiotic communities (pathogenic dental plaque biofilm), while necessary, are not sufficient to precipitate periodontitis [9]. This is because periodontal tissue destruction is predominantly mediated by the host inflammatory response to the microbial challenge [10–12]. This understanding has provided a strong rationale for developing strategies that modulate the host microenvironment to treat periodontitis. Such novel approaches can be used in an adjunctive mode to enhance current therapies (*e.g.*, mechanical removal of the dental plaque biofilm), which are not always adequate to control periodontitis [13–15]. Indeed, periodontitis continues to be a serious public health issue with a substantial economic burden [2, 16, 17].

Therefore, the identification of key inflammatory pathways that drive periodontal tissue destruction has important translational implications and hence received particular attention in the past decade. In this context, we discuss recent proof-of-concept studies in preclinical models that have established a causal relationship between periodontitis and complement, thereby supporting the usefulness of complement-targeted therapies in this oral disease.

2. Complement and periodontitis

Complement represents a network of interacting fluid-phase and cell surface-associated molecules that activate, amplify, and regulate immune and inflammatory response pathways [18]. The integrated complement system includes the classic serum proteins (C1-9), pattern-recognition molecules, convertases and other proteases, regulators, and receptors for interactions with immune mediators, which together coordinate the host response to infection or tissue injury [18]. For instance, complement-based interactions can amplify host immune and inflammatory responses through synergy with Toll-like receptors (TLRs) [19–21], provide a barrier against the spread of infections by potentiating local clotting [22, 23], and regulate the activation and differentiation of B cells and T-cell subsets [24–28]. The complement cascade can be initiated by distinct mechanisms (classical, lectin, or alternative), all of which converge at C3, the third complement component, leading to the generation of cleavage products that mediate various functions. For instance, convertase-mediated cleavage of C3 and C5 generates, respectively, the anaphylatoxins C3a and C5a which activate specific G-protein-coupled receptors (C3aR and C5aR1 [CD88]) that not only mediate recruitment and activation of inflammatory cells but also cross-talk with TLR

signaling pathways [20]. Moreover, other molecules, such as C4b and C3b, act as opsonins promoting microbial opsonization and phagocytosis, whereas the so-called terminal pathway culminates in the C5b-9 membrane attack complex which can directly lyse susceptible targeted microbes [18] (Figure 1A).

The possible involvement of complement in human periodontitis was first recognized in the 1970s and 1980s by clinical studies that associated the disease with the presence of complement activation fragments [29–35]. Moreover, successful periodontal therapy (*i.e.*, that reduced clinical indices that measure periodontal inflammation and tissue destruction) resulted in decreased C3 activation in the gingival crevicular fluid (GCF) [36]. Conversely, in an experimental gingivitis study in human volunteers, the progression of gingival inflammation was correlated with increased C3 cleavage in the GCF [37]. These early studies therefore suggested that complement is involved in the pathogenesis of periodontal disease, thereby constituting a potential therapeutic target. Fortunately, complement-targeted drug discovery has resulted in various inhibitors that can be used in experimental and clinical applications.

3. Therapeutic complement inhibitors

The increasing awareness that complement is heavily involved in the pathogenesis of a growing list of inflammatory or degenerative diseases has prompted systematic efforts to develop potent and highly selective complement inhibitors with potential for clinical translation [38, 39]. The first clinically approved complement-targeted drugs, anti-C5 (Eculizumab; Soliris, Alexion) and C1-inhibitor (C1-INH; various manufacturers) are now used for the treatment of paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS), and for hereditary angioedema, respectively [40]. Additional candidate drugs for modulating complement activity at various points of the cascade are under development ranging from large biological biomolecules (antibodies and engineered proteins) to interfering RNA, aptamers and low-molecular-weight inhibitors and antagonists [38, 41, 42]. With close to 50 molecules involved in the cascade, the complement system offers numerous potential targets for pharmacological interference. Among those, C3 is attractive due to its strategic position in the complement cascade, forming a central ‘hub’ that relays initiating signals to downstream activation of effector molecules and rapid amplification of complement responses [41]. Especially in diseases with complex involvement of various pathways or those driven by the alternative pathway, C3 interception is therefore an appropriate choice that can afford strong and broad complement inhibition.

The members of the compstatin family of C3 inhibitors are so far the only small-size clinical drug candidates that act directly on C3 [40]. The original compstatin is a 13-residue cyclic peptide that selectively binds to native C3 as well as to its bioactive fragments C3b, iC3b and C3c [43]. It prevents the convertase-dependent cleavage of C3, thereby blocking complement activation at the most central point of this cascade [38]. The crystal structure of compstatin in complex with its binding partner C3c revealed that compstatin sterically hinders the interaction of native C3 with the C3 convertases [44, 45]. On the basis of the structural relationship between compstatin and C3, intensive optimization efforts resulted in

improved compstatin derivatives with enhanced inhibitory potency, target binding affinity and pharmacokinetic parameters. Importantly, both the parental compstatin and its derivatives exhibit narrow species specificity as they bind exclusively to C3 from humans and non-human primates [46], thereby precluding their use in lower animal models.

The compstatin derivative Cp40 has emerged as particularly promising clinical candidate for systemic applications due to its subnanomolar affinity for C3 ($K_D = 0.5$ nM) and a plasma half-life that exceeds expectations for most peptidic drugs [40, 47]. These unique pharmacokinetic properties of Cp40 were accounted for by a ‘target-driven’ model, wherein an initial rapid clearance of excess free (*i.e.*, not C3-bound) peptide is followed by slow clearance of C3-bound peptide. Since the measured half-life values of distinct compstatin analogs correlate with their C3-binding affinities [47], it can be concluded that the tight binding of Cp40 to C3 delays its clearance. Cp40 has been licensed by Amyndas Pharmaceuticals and formed the basis for developing the clinical candidate drug AMY-101, intended for therapeutic intervention in complications of ABO-incompatible kidney transplantation, C3 glomerulopathy (C3G), PNH, and periodontitis [39]. It is worth noticing that AMY-101 has recently received orphan designation for PNH and C3G from the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA). In addition, an earlier compstatin analog (APL-1 and its PEGylated form APL-2; Apellis Pharmaceuticals) is clinically developed for use in age-related macular degeneration, PNH and chronic obstructive pulmonary disease (COPD).

4. Complement inhibition in rodent models of periodontitis

As mentioned above, there are strong clinical indications for an association of complement with periodontal disease. However, cause-and-effect relationships cannot be typically addressed in human studies, most of which are correlative. In this regard, relevant animal models have to be used to test causative links between candidate mechanisms and disease [48], thereby confirming therapeutic targets and paving the way to human clinical trials. As the complement response is intertwined with TLR activation, the two systems are discussed together in the mechanistic studies presented below.

In response to infection, tissue injury or other types of insults, complement and TLRs are rapidly activated, often by the same ligands; for instance, lipopolysaccharide (LPS; a TLR4 agonist), zymosan (TLR2/6 agonist) and CpG DNA (TLR9 agonist) can activate complement in addition to initiating TLR signaling [19]. In this regard, systemic administration of different TLR agonists to mice lacking decay-accelerating factor (DAF), a major membrane-associated complement inhibitor, elicits significantly higher plasma levels of pro-inflammatory cytokines relative to wild-type controls [20]. Consistently, remarkably high plasma levels of pro-inflammatory cytokines are also elicited in mice systemically co-injected with TLR agonists and cobra venom factor, a potent complement activator [20]. These observations suggested that complement can amplify TLR-mediated inflammatory responses and further work has conclusively implicated C3aR and C5aR1 in a synergistic cross-talk with TLR pathways [20] (Figure 1). Accordingly, the concomitant activation of C5aR1 and TLR2 by local co-injection of specific agonists (C5a and Pam3Cys) in the gingiva of mice resulted in the induction of significantly higher levels of TNF, IL-1 β , IL-6,

and IL-17A than activation of each receptor alone [49]. These data, taken together with findings that mice lacking either C5aR1 or TLR2 are resistant against inflammatory periodontal bone loss [50, 51] suggested that periodontal inflammation depends in great part on a synergy between complement and TLRs.

Consistently, in a mouse model of periodontitis where the disease is initiated by dysbiosis caused by oral inoculation with *Porphyromonas gingivalis* [48], local treatment with a C5aR1 antagonist (PMX-53) inhibited periodontal inflammation (TNF, IL-1 β , IL-6, and IL-17) and bone loss, regardless of the presence of TLR2 (*i.e.*, inflammatory bone loss can be effectively inhibited by blocking just one of the two cross-talking receptors) [49]. However, in this model, C5aR1 is also a target of immune subversion by *P. gingivalis* (which actually can directly activate C5aR1 through its arginine-specific gingipains that can cleave C5 to generate high local concentrations of C5a [50, 52, 53]) leading to the dysbiotic transformation of the microbiota [54] (Figure 1B). Thus, it was uncertain whether C5aR1 blockade prevented dysbiosis or inflammation or both. Therefore, PMX-53 was also tested in a model of periodontitis, where the disease is induced independently of *P. gingivalis*, which is not a natural constituent of the murine oral microbiota. Specifically, ligature-induced periodontitis involves the placement of silk ligature around molar teeth resulting in massive local bacterial accumulation and rapid induction of severe bone loss in specific-pathogen-free (but not germ-free) animals [55–57]. Although inflammation and bone loss was rapidly induced (within 5 days) at the ligated sites of control mice, mice locally treated with PMX-53 at the ligated sites were significantly protected from inflammation and bone loss [49]. In a similar ligature-induced periodontitis model in rats, an independent study administered PMX205 (an analog of PMX53) via the drinking water. This treatment also suppressed bone loss albeit with reduced efficacy [58] probably owing to the different routes of drug administration and/or to the use of different animal species.

Although *P. gingivalis* can readily colonize the periodontium of C3-deficient mice, the resulting dysbiosis is transient and the periodontal microbiota cannot be sustained at high levels throughout the experimental period (6 weeks) as observed in wild-type controls [59]. Moreover, *P. gingivalis*-colonized C3-deficient mice have significantly less inflammation and bone loss than *P. gingivalis*-colonized wild-type mice [59]. Similarly, C3-deficient mice are protected from ligature-induced periodontitis and aging-associated periodontitis, which develops naturally as a function of old age [59]. These studies therefore established that C3 is critical for inflammatory bone loss in different models of murine periodontitis.

The reliability of mouse models for the investigation of human inflammatory diseases has been questioned by a study that examined gene expression profiling of C57BL/6J mice and humans during endotoxemia, suggesting poor correlation between the human genes and mouse orthologs and vice versa [60]. Whether this notion can be broadened to include different inflammatory diseases is uncertain. In fact, such shortcoming may not be applicable to periodontal disease, since the same inflammatory mediators (*e.g.*, TNF, IL-1 β , prostaglandin E2) were shown to mediate inflammatory bone loss across different species, including mice, rats, dogs, non-human primates, and humans [61–66]. Nevertheless, it is important to test potential new treatments in the most relevant preclinical models for increasing the chances that candidate drugs can be protective also in humans. Moreover,

certain drugs require the use of higher animals since they lack specificity for widely used smaller animals, such as rodents. In this regard, the C3 inhibitor compstatin could not be tested in mice due to its exquisite specificity for primate C3 [38, 40].

5. Complement inhibition in non-human primate periodontitis

The immune system and periodontal anatomy of cynomolgus monkeys are similar to those of humans, and periodontitis in these animals exhibits clinical, microbiological, and immuno-histological features that are highly similar to those observed in human periodontitis [67–71]. Therefore, the cynomolgus model is considerably more predictive of drug efficacy in humans compared to widely used models, such as those in rodents, rabbits, or dogs.

As alluded to above, genetic studies in mice implicated C3 in the pathogenesis of periodontitis, in line with earlier correlative studies in humans. The appropriateness of C3 as a therapeutic target in periodontitis was assessed in cynomolgus monkeys (*Macaca fascicularis*) using the compstatin analog Cp40 [59].

To induce periodontitis in young cynomolgus monkeys, silk ligatures were placed around posterior teeth on both halves of the lower jaw (mandible) for 6 weeks. Cp40 treatment was initiated three days after ligature placement using a split-mouth experimental design, where each animal serves as its own control. Specifically, one side was treated with Cp40 and the other side with an inactive control peptide (i.e., sequence-scrambled compstatin analog). The disease was monitored clinically by analyzing indices that measure periodontal inflammation and tissue destruction, according to the criteria of the American Academy of Periodontology [72]. Cp40 caused a significant inhibition of gingival index and clinical attachment loss and these effects correlated with lower GCF levels of proinflammatory cytokines (e.g., TNF, IL-1 β , IL-17) and RANKL, a key osteoclastogenic factor, as well as with reduced osteoclastogenesis in bone biopsy specimens [59]. Accordingly and importantly, radiographic analysis of the ligated teeth revealed that Cp40-treated sites developed significantly less bone loss than control-treated sites [59]. Because the GCF levels of osteoprotegerin (OPG), a natural inhibitor of RANKL, were sustained at higher levels in Cp40-treated than the control, Cp40 caused a favorable reversal of the RANKL/OPG ratio, which is a useful indicator of human periodontitis [73].

In a follow-up study, the objective was to determine whether Cp40 can also be effective in a therapeutic setting, that is, when the drug is delivered after disease has been established. Specifically, the goal was to test whether Cp40 could inhibit pre-existing chronic periodontitis in adult cynomolgus monkeys. Chronic periodontitis typically affects adults, and aging is associated with increased prevalence and severity of periodontitis, which might be attributed, in part, to alterations of the immuno-inflammatory status of the periodontal tissue [74]. This hypothesis is consistent with recent studies in rhesus monkeys showing age-dependent differential expression of immune and inflammatory genes in the periodontium [75, 76]. Cynomolgus monkeys with naturally-occurring periodontitis (selected by screening a population of adult animals in the Simian Conservation Breeding and Research Center; Makati, Philippines) were locally injected in the gingiva with Cp40 either once a week (5

animals) or three times per week (10 animals) for six weeks followed by a 6-week follow-up period without Cp40 treatment. Clinical examination and collection of GCF were performed at baseline and throughout the experimental period. Whether given once or three times weekly, Cp40 caused a significant reduction in clinical indices that measure periodontal inflammation (gingival index and bleeding on probing), tissue destruction (probing pocket depth and clinical attachment loss) or tooth mobility often associated with bone loss. The reduction in clinical indices correlated with decreased GCF levels of proinflammatory and bone-resorptive mediators (*e.g.*, IL-17 and RANKL) and decreased numbers of osteoclasts in bone biopsies. The protective effects of Cp40 persisted, albeit at reduced efficacy, for at least six weeks following drug discontinuation. Therefore, Cp40 can reverse pre-existing chronic periodontal inflammation in the absence of additional treatments, such as scaling and root planing, thereby paving the way to a novel anti-inflammatory therapy for the treatment of human periodontitis [77].

As discussed above, complement pathways (*e.g.*, C3aR or C5aR1 signaling) cross-talk with and amplify TLR-mediated inflammatory responses in circulation and various tissues [19, 20] including the periodontium [49]. C3 inhibition in periodontitis therefore may also suppress inflammation that is initiated by TLR activation in response to microbial ligands such as lipopolysaccharide, lipoproteins, and bacterial DNA [78, 79]. In addition, since TLR activation can be triggered by endogenous TLR ligands (*e.g.*, biglycan, hyaluronan fragments, and heparan sulfate fragments) that are released upon tissue injury [80, 81], complement inhibition may also contribute to blocking the progression of periodontitis.

In human monocytes, C3a regulates the release of intracellular ATP to the extracellular milieu, thereby controlling the activation of the NLRP3 inflammasome and the ensuing secretion of IL-1 β , which in turn promotes CD4⁺ T cell production of IL-17 [82]. Interestingly in this context, a clinical study revealed elevated expression of NLRP3 (NALP3) inflammasome in periodontal disease correlating with augmented IL-1 β expression [83]. Moreover, the formation of sublytic membrane attack complex on human epithelial cells induces intracellular Ca²⁺ fluxes leading to NLRP3 inflammasome activation and IL-1 β release [84]. Both of these complement-dependent mechanisms can be blocked by Cp40, potentially accounting – at least in part – for the reduced IL-1 β and IL-17 levels in monkey periodontitis upon Cp40 treatment.

6. Clinical considerations of complement inhibition in periodontitis

Given the microbial component of the disease, therapeutic complement inhibition may not appear as intuitive treatment option for periodontal disease. Yet, the combined studies presented above clearly suggest a clinical value of blocking complement, and in particular the central component C3, as it directly affects inflammation and may, potentially, help reversing dysbiosis. In this regard, compstatin Cp40 has already shown promising efficacy in non-human primate models [59, 77], which may pave the way to clinical trials. There are still aspects that need to be carefully considered in this context, such as questions concerning administration frequency and route, dosing, and selection of patients that would benefit most from such a treatment.

One concern often raised for therapeutic use of complement inhibitors, including C3-targeted compounds, is whether long-term complement blockade may affect the competency of antimicrobial defenses. At present, however, there is limited clinical experience regarding potential adverse effects of long-term systemic anti-C3 therapy [40, 85]. Individuals with primary C3 deficiencies have increased risk of pyogenic infections mainly in the early years of life, but this increased susceptibility typically subsides in adulthood, probably due to the emergence of compensatory defense mechanisms [86]. It should also be noted that complement inhibition at C3 does not interfere with C4b-mediated opsonization of bacteria via the classical and lectin pathways [87]. Importantly, C3 interception using small-molecule inhibitors, such as compstatin, can be readily phased out if necessary, thereby enabling swift recovery of C3's opsonic activity during an infection. Accumulated experience from approved anti-complement drugs (*e.g.*, eculizumab) indicate that vaccination against encapsulated bacteria (*e.g.*, meningococci) can largely mitigate infectious risks. In case of C3-directed therapy, additional vaccinations and prophylactic use of antibiotics may be included to promote a safe use in chronic settings. On the other hand, in acute conditions that require transient C3 interception, as in hemodialysis [88], C3 inhibitors are not likely to increase the risk of infection and may not require vaccination. As C3 inhibitors enter clinical trials, more definitive clinical experience will be obtained regarding their safety.

Importantly for periodontitis, these potential safety concerns are not expected to be relevant in the treatment of this disease. Systemic exposure with Cp40 after local injection into the gingival tissue is considered minimal and should not affect complement activity in circulation or other tissues. In this regard, it should be noted that C3 is the most abundant complement protein in blood (1.0 to 1.5 mg/ml); therefore, small amounts of Cp40 that may 'escape' from the gingiva should be readily bound by excess C3 in blood, hence not reaching other tissues at sufficient active concentrations for local complement inhibition. In the treatment regimen used by Maekawa et al [77], a total of 1.5 mg Cp40 was injected (15 sites at 100 µg/site). Even if the full intra-gingival dose were administered systemically rather than locally, this would only amount to 0.2–0.3 mg/kg bodyweight, thereby not reaching Cp40 levels in excess of C3 that could penetrate to other sites; indeed, in non-human primates, a Cp40 dose of 1–2 mg/kg bodyweight was required to reliably achieve target-exceeding drug levels after systemic administration [89]. These considerations are fully consistent with experimental evidence. Indeed, gingival inflammation in the mandible of all animals of the Maekawa et al study was not significantly affected during the course of the local Cp40 treatments in the maxilla, in contrast to the maxillary gingiva where inflammation was significantly inhibited [77]. Furthermore, in an earlier split-mouth design study, the Cp40-treated gingival sites exhibited significantly less inflammation than the contralateral gingival sites that were treated with control peptide [59].

It should also be noted that C3 blockade in periodontitis is unlikely to lead to uncontrolled local microbial growth. This notion is based on findings from the mouse model, where C3-deficient mice have reduced periodontal bacterial burden relative to C3-sufficient controls in the course of experimental periodontitis [59]. These data suggest that defective complement activation does not predispose to defective immune surveillance in the periodontium and are consistent with the notion that inflammation and dysbiosis engage in a positive feedback loop (Figure 1B). Specifically, inflammation generates tissue breakdown products (*e.g.*,

degraded collagen peptides or heme-containing compounds) that are used as a food source by periodontitis-associated bacteria, thereby exacerbating dysbiosis [90, 91]. Therefore, complement inhibition can potentially block the overgrowth of the dysbiotic microbiota. In this regard, the control of inflammation in mouse and rabbit models of periodontitis additionally decreases the bacterial load [65, 66, 92, 93]. Conversely, and consistently, the bacterial biomass of human periodontitis-associated biofilms increases with increasing inflammation [94]. The notion that inflammation can promote bacterial growth has been observed also in other settings involving distinct mechanisms. For instance, IL-1 β , IL-6 and TNF were shown to enhance the growth and virulence potential of certain pathogens that bind these cytokines through specific receptors [95–97].

7. Conclusions and Outlook

Although Cp40 was successfully applied as a stand-alone treatment for ligature-induced as well as naturally-occurring periodontitis in non-human primates, the drug is more likely to find application as an adjunctive therapy to the management of human periodontitis. Future clinical trials could investigate the potential of Cp40 to inhibit periodontal inflammation and bone loss compared to scaling and root planing, whereas in very severe cases of the disease, Cp40 could be combined with scaling and root planing and compared to periodontal surgery, in an effort to obviate the need for a surgical approach. It should be noted that future host-modulation interventions, such as Cp40, may not only necessarily be implemented in a therapeutic setting but could also be provided on a preventive basis to high-risk individuals before the onset of periodontitis, such as cigarette smokers and diabetic patients [98, 99]. Although the mechanisms by which Cp40 blocks periodontal inflammation are largely understood and were discussed above, the optimal frequency of its administration for long-term treatment of human periodontitis is currently uncertain and may need to be determined empirically. However, it is encouraging that the protective effects of Cp40 are maintained for at least six weeks following its withdrawal from treated monkeys [77]. As discussed in the previous section, the tight binding of Cp40 to C3 is expected to delay its clearance [47]. Similarly, the expected tight binding of Cp40 to abundant C3 in the inflamed periodontium could contribute to delayed elimination of the drug from the tissues, in turn accounting—at least in part—for the sustained protective effect of Cp40 in monkeys with pre-existing natural periodontitis [77]. Alternatively, or additionally, the observed inhibition of inflammation by Cp40 may shift the balance towards tissue homeostasis, which might resiliently restrain for some time pathological processes even in the absence of the drug. As discussed above, AMY-101, a clinically developed Cp40-based drug is currently under evaluation as a potential treatment of complications of ABO-incompatible kidney transplantation, C3G, and PNH [40]. The possibility that AMY-101 can find application for the treatment of human periodontitis merits investigation in future clinical trials given the impressive results from two different models of monkey periodontitis.

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Highlights

- Complement is overactivated in periodontitis, a dysbiotic oral inflammatory disease
- C3, C3a receptor, and C5a receptor 1 (CD88) mediate periodontitis in rodents
- C3 inhibition blocks periodontitis in non-human primates
- C3 inhibitors (Cp40/AMY-101) are considered for treatment of human periodontitis

Figure 1. Complement and its involvement in periodontal dysbiosis and inflammatory bone loss

A) Complement cascade: The classic, lectin, and alternative pathways converge to activate C3 leading to the generation of effector molecules. These include the inflammatory anaphylatoxins C3a and C5a, which respectively activate C3aR and C5aR1, which moreover cross-talk with TLRs. Intriguingly, the keystone periodontal pathogen *P. gingivalis* can directly activate C5aR1 through its arginine-specific gingipains that can cleave C5 to generate biologically active C5a. C3b is an opsonin that promotes microbial opsonization. The cleavage of C5 by its convertase (C3bBb3b) also generates C5b which in the terminal pathway initiates the assembly of the C5b-9 membrane attack complex (MAC), which induces lysis of susceptible targeted microbes. The alternative pathway C3 convertase, C3bBb, is also involved in an amplification loop for all complement pathways. Compstatin and derivative drugs, such as Cp40, block C3 activation, thus inhibiting all activities downstream of C3. **B) Dysbiotic inflammation:** C5aR1 is involved a subversive crosstalk with Toll-like receptors (TLR) leading to the remodeling of a symbiotic microbiota into a

dysbiotic one. This cross-talk is instigated by keystone pathogens (see text for details). The resulting dysbiotic microbial community causes inflammation that is largely dependent on complement (C3aR, C5aR1)-TLR crosstalk. Inflammation and dysbiosis reinforce each other since inflammatory tissue breakdown products are used as nutrients by the dysbiotic microbiota, which thus further exacerbates inflammation and ultimately leads to bone loss, the hallmark of periodontitis. Therapeutic blockade of C3 activation/cleavage using Cp40 has blocked periodontitis in non-human primates.