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C3-targeted host-modulation approaches to oral inflammatory conditions

Tetsuhiro Kajikawa [a,b], Dimitrios C. Mastellos [c], Hatice Hasturk [d], Georgios A. Kotsakis [e], Despina Yancopoulou [f], John D. Lambris [g], George Hajishengallis [a,*]

[a] University of Pennsylvania, Penn Dental Medicine, Department of Basic and Translational Sciences, Philadelphia, PA, USA
[b] Tohoku University Graduate School of Dentistry, Department of Periodontology and Endodontology, Sendai, Miyagi, Japan
[c] National Center for Scientific Research 'Demokritos', Division of Biodiagnostic Sciences and Technologies, INRASTES, Athens, Greece
[d] The Forsyth Institute, Center for Clinical and Translational Research, Cambridge, MA, USA
[e] University of Texas Health Science Center at San Antonio, School of Dentistry, Department of Periodontics, San Antonio, TX, USA
[f] Amyndas Pharmaceuticals, Glyfada, Greece
[g] University of Pennsylvania, Perelman School of Medicine, Department of Pathology and Laboratory Medicine, Philadelphia, PA, USA

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ABSTRACT

Periodontitis is an inflammatory disease caused by biofilm accumulation and dysbiosis in subgingival areas surrounding the teeth. If not properly treated, this oral disease may result in tooth loss and consequently poor esthetics, deteriorated masticatory function and compromised quality of life. Epidemiological and clinical intervention studies indicate that periodontitis can potentially aggravate systemic diseases, such as, cardiovascular disease, type 2 diabetes mellitus, rheumatoid arthritis, and Alzheimer disease. Therefore, improvements in the treatment of periodontal disease may benefit not only oral health but also systemic health. The complement system is an ancient host defense system that plays pivotal roles in immunosurveillance and tissue homeostasis. However, complement has unwanted consequences if not controlled appropriately or excessively activated. Complement overactivation has been observed in patients with periodontitis and in animal models of periodontitis and drives periodontal inflammation and tissue destruction. This review places emphasis on a promising periodontal host-modulation therapy targeting the complement system, namely the complement C3-targeting drug, AMY-101. AMY-101 has shown safety and efficacy in reducing gingival inflammation in a recent Phase 2a clinical study. We also discuss the potential of AMY-101 to treat peri-implant inflammatory conditions, where complement also seems to be involved and there is an urgent unmet need for effective treatment.

1. Introduction

Periodontitis is a prevalent inflammatory disease that affects approximately 50% of human adults, whereas the severe form of the disease afflicts 10% of the adult population [1]. Inflammation triggered by dysbiotic microbial communities in subgingival sites of the teeth may lead to degradation of the gingival connective tissue and resorption of the alveolar bone that supports the teeth [2]. Thus, if periodontitis is not controlled, it may lead to tooth loss. As a consequence, patients with periodontitis often show impaired mastication and poor esthetics, which erodes their quality of life. The estimated direct cost due to periodontal disease in the United States and Europe in 2018 was $3.49 billion and €2.52 billion respectively [3]. The estimated indirect cost that includes also the productivity losses because of absenteeism from work was $150.57 billion in the United Sates and €156.12 billion in Europe. Thus, periodontal disease remains both a serious economic and public health burden [3]. In the latter regard, periodontitis is moreover associated with increased risk for certain systemic diseases, such as, cardiovascular disease, diabetes mellitus, rheumatoid arthritis and Alzheimer disease [4]. Current standard-of-care periodontal therapy (aiming to remove the disease-causing biofilm by mechanical debridement occasionally with adjunctive anti-microbial approaches) is not always effective, particularly in highly susceptible individuals [5]. Therefore, there is an unmet need for effective host-modulation therapies as an adjunct to the standard treatment of periodontitis.

The complement system is an ancient immune-surveillance system that has pivotal roles in protection from pathogen invasion and tissue homeostasis [6]. More than three decades ago, independent studies

* Correspondence to: University of Pennsylvania, Penn Dental Medicine, 240 South 40th Street, Philadelphia, PA 19104-6030, USA.
E-mail address: geoh@upenn.edu (G. Hajishengallis).

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detected increased abundance of complement activation fragments in the gingival crevicular fluid (GCF) and in gingival tissue isolated from patients with periodontal inflammation, relative to healthy controls [7–12]. These studies provided correlative evidence for the importance of the complement system in the pathogenesis of periodontitis or gingivitis, a reversible form of gingival inflammation that precedes the onset of periodontitis. Subsequently, cause-and-effect and mechanistic studies in mouse and non-human primate (NHP) models of periodontitis showed that complement overactivation indeed drives and aggravates periodontal inflammation [13–15].

The complement cascade comprises three distinct pathways, the classical, lectin and alternative pathways, which all converge at the third complement component (C3) [16]. Given that no particular complement initiation pathway has been exclusively implicated in periodontitis and that C3-deficient mice are protected from periodontitis [17], it was quite reasonable to target C3 to ameliorate periodontal inflammation and disease. In this context, the peptidic C3 inhibitor compstatin was originally discovered in 1996 [18] and, in subsequent years, new and improved analogs were developed with increased affinity for C3, enhanced plasma residence and solubility and more favorable pharmacokinetic profiles [19–21]. Importantly, the third-generation compstatin, designated AMY-101, was recently locally administered to patients with periodontal inflammation under a Phase 2a clinical trial exhibiting promising outcomes [22]. In this review, we discuss this emerging periodontal therapy targeting C3. Additionally, we propose that AMY-101 may have therapeutic effects on peri-implantitis, an inflammatory condition that affects the tissues around dental implants, leading to progressive loss of supporting alveolar bone [23].

2. The role of complement in periodontitis

The complement system plays pivotal roles in immune responses. It was discovered in the late 19th century as a heat-labile antimicrobial entity [24] and, since then, some 50 proteins have been identified as molecules involved in the core complement cascades [6]. Although the complement system critically contributes to immune surveillance and tissue homeostasis [25], complement pathways are, under certain conditions, dysregulated and produce an aberrant amount of inflammatory mediators, such as anaphylatoxins [6]. For instance, it has been proven that the dysregulation of the complement system results in development of specific diseases, i.e., atypical hemolytic uremic syndrome (aHUS), C3 glomerulopathy (C3G), and paroxysmal nocturnal hemoglobinuria (PNH), depending on the dysregulated point in the complement cascades [26]. Moreover, recent evidence indicates that complement overactivation is associated with the maladaptive host inflammatory response to SARS-CoV-2 that inflicts tissue damage in multiple organs of patients with coronavirus disease 2019 (COVID-19) [27–30].

The periodontal tissue is constantly exposed to indigenous bacteria that form biofilms on the tooth surfaces. If the biofilm is not removed properly by daily tooth brushing or periodic professional tooth cleaning, the microorganisms flourish and their interplay with ensuing inflammation leads to dysbiosis of the biofilm which further aggravates inflammation. A persistent dysbiotic biofilm on tooth root surfaces causes destructive inflammation leading to deepened periodontal pockets and alveolar bone resorption [2,31].

The potential involvement of complement in periodontitis was supported by early clinical studies that associated increased complement activation in GCF or periodontal tissue with periodontal disease activity [7–12]. Consistently, a recent study has shown that the concentration of total C3 and its cleavage product C3c in saliva is higher in patients with periodontitis as compared to that in healthy controls [32]. Moreover, the same study suggested that both C3 and C3c could be used to differentiate periodontal disease from periodontal health [32]. The authors further speculated that the increased C3 levels might be due to the increase of vascular permeability by periodontal inflammation and/or the increased local production of C3 (and other complement proteins) by activated immune cells (*e.g.*, macrophages and dendritic cells) in the periodontal tissue. The same group subsequently showed that patients with grade C periodontitis (i.e., with high risk of further progression) have increased levels of both C3c and C3dg cleavage products in their saliva than healthy controls [33]. Interestingly, liquid chromatography–mass spectrometry (LC–MS) analysis using GCF harvested from NHPs with naturally occurring periodontitis revealed involvement of both the alternative ("complement activation, alternative pathway (GO:0006957)") and classical pathway ("complement activation, classical pathway (GO:0006958)") of complement activation, although the alternative pathway was the most enriched of all identified biological pathways [34]. Given that human and NHP periodontitis share similar clinical and immunological features, this study further supported the notion that the complement system is involved in human periodontal disease pathogenesis. Consistent with a link between the classical pathway and periodontal tissue homeostasis, almost all patients who are diagnosed with periodontal Ehlers-Danlos syndrome, an autosomal-dominant disorder caused by gene mutations in the classical pathway components C1R or C1S, suffer from early-onset periodontitis, although the underlying molecular mechanism remains unclear [35]. A recent study showed that polymorphisms in the complement factor H, a soluble complement regulator essential to control the alternative pathway, are associated with clinical and radiographic signs of periodontitis [36]. These reports suggest an association between the complement system and periodontal health and disease.

To conclusively demonstrate a cause-and-effect relationship between complement activation and periodontitis, several mechanistic studies were carried out in animal models. Mice housed in specific-pathogen-free conditions can develop naturally occurring chronic periodontal bone loss with ageing [37,38]. Intriguingly, both C3a receptor- and C5a receptor 1- deficient mice showed significantly less alveolar bone loss at 18 months of age compared to age-matched wild-type (WT) mice [13]. Similarly, the periodontal bone loss in 6- or 9-month-old C3-deficient mice was significantly diminished compared to that of age-matched WT mice [14]. The C3-deficient mice were also resistant to alveolar bone loss induced either by ligature-induced periodontitis, a widely utilized model for periodontitis [39], or by oral gavage with the human periodontal pathogen *Porphyromonas gingivalis* [14]. Taken together, these studies in mouse models suggest that the complement system is causally connected to periodontitis, thereby arguing for a complement-targeted therapeutic approach to this oral disease (Fig. 1).

3. The application of C3 inhibitor, AMY-101, in human periodontal inflammation

Since C3 plays a central role in complement activation initiated by all three pathways (classical, lectin and alternative) and periodontitis is not associated exclusively with a specific initiation pathway, targeting C3 should ameliorate periodontal inflammation efficiently. Under this rationale, two studies have used a C3 inhibitor (the third-generation compstatin analog Cp40) for local administration in the gingiva of NHPs which were subjected to ligature induced-periodontitis or had naturally occurring periodontitis [14,40]. In both settings, Cp40 not only improved clinical indices that measure periodontal inflammation (i.e., clinical attachment level, gingival index, bleeding on probing, mobility index), but also significantly reduced the amount of inflammatory cytokines (e.g., IL-1β, IL-6, IL-8, IL-17A) in the GCF [14,40]. In the ligature-induced periodontitis model, which also assessed the degree of bone resorption in treated and untreated sites, Cp40 was shown to inhibit alveolar bone loss [14].

The safety and efficacy of locally delivered Cp40 to treat naturally occurring periodontitis in NHPs was thoroughly examined and confirmed in subsequent studies. A dose of 2 mg/mL of Cp40 was shown to alleviate periodontal inflammation without causing local irritation [41]. Importantly, Cp40 showed efficacy even when given once every 3 weeks and the therapeutic benefits persisted for at least 6 weeks after

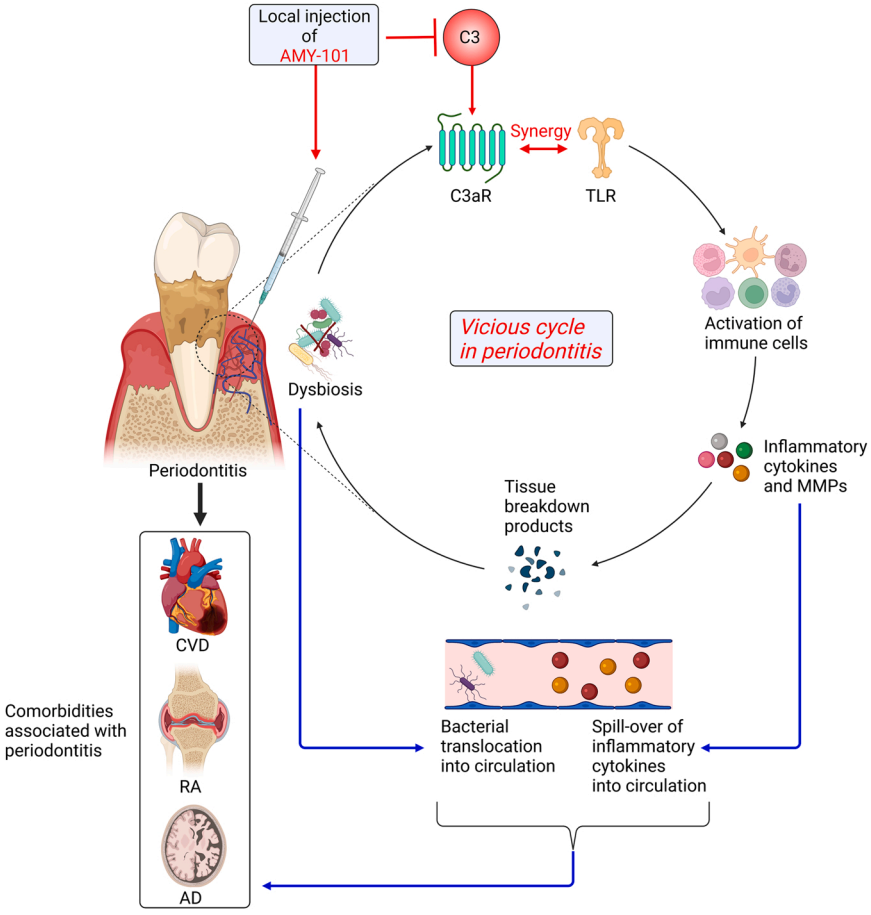

Fig. 1. A vicious cycle in periodontitis induced by complement activation and potential mechanisms linking periodontitis to comorbid inflammatory conditions. Periodontal disease pathogenesis represents a vicious cycle where complement signaling has a crucial role. Dysbiosis in the periodontium triggers both complement and TLR signaling. These signaling pathways can synergistically activate immune cells resulting in production of inflammatory cytokines and MMPs. MMPs degrade periodontal tissue and the tissue breakdown products provide nutrients for bacterial growth (e.g., degraded collagen provides a source of amino acids for asaccharolytic and proteolytic pathobionts), thereby further promoting and perpetuating dysbiosis. Bacterial translocation and spillover of inflammatory cytokines into circulation can occur in this vicious cycle, resulting in systemic inflammation that underlies the connection of periodontitis to its comorbidities, such as CVD, RA, and AD. By blocking the local destructive inflammatory response that fuels dysbiosis and also increases the systemic inflammatory burden, locally administered AMY-101 can break this disease-provoking cycle. Therefore, AMY-101 may have benefit not only in treating periodontitis, but also improving the status of comorbidities of periodontitis. AD, Alzheimer's disease; C3aR, C3a receptor; CVD, cardiovascular disease; MMP, matrix metalloproteinase; RA, rheumatoid arthritis; TLR, Toll-like receptor.

treatment completion [41]. Of note, the total amount of Cp40 injected locally into the NHP gingiva was 0.2–0.3 mg/kg bodyweight, whereas 1–2 mg/kg of systemically administered Cp40 is required to achieve target-exceeding drug concentration [42]. Therefore, any amount of Cp40 that may diffuse into the blood circulation after local administration is likely bound by excess C3 in the blood. Moreover, systemic inhibition of C3 by 2 mg/kg of Cp40 in NHPs was not associated with a weakened immune system or susceptibility to infections [43]. Together, these efficacy and safety studies in NHPs provided proof-of-concept for using this complement C3 inhibitor to treat human patients with periodontal inflammation.

Cp40 is clinically developed as 'AMY-101' for the treatment of various complement-mediated diseases by Amyndas Pharmaceuticals [44]. A phase 1 trial of AMY-101 was conducted in 2017 to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of AMY-101 (NCT03316521) [17]. This study, which enrolled 50 healthy male volunteers, revealed that the drug was safe and well tolerated without significant adverse events [17]. In 2019, the Food and Drug Administration (FDA) approved an Investigational New Drug application of AMY-101 as a phase 2a study to assess its safety and efficacy in adults with periodontal inflammation (gingivitis) (NCT03694444) [17]. The study was performed at the Forsyth Institute and 40 individuals with periodontal inflammation were selected as study participants following screening of 98 patients [22]. AMY-101 was applied locally by intragingival injections at baseline, day 7 and day 14 for evaluating both safety and efficacy. No drug-related serious systemic adverse events, which could result in the study discontinuation of participants, were reported. Importantly, AMY-101 caused significant reductions in two key clinical indices – modified gingival index score and bleeding on probing – which reflect the severity of gingival inflammation. Moreover, the drug caused a significant reduction in the GCF levels of matrix metalloproteinase (MMP)-8 and MMP-9, which are biomarkers of periodontal tissue destruction [45,46]. These therapeutic effects persisted for at least 90 days after the treatment started [22], reminiscent of the prolonged anti-inflammatory effect that was seen in the NHP studies [40,41]. This auspicious result merits further investigation in a multi-center phase 3 clinical trial that will evaluate AMY-101 as an adjunctive therapeutic to treat patients with periodontal inflammation.

4. AMY-101 treatment in periodontitis and associated comorbidities

Although periodontitis is a chronic inflammatory disease that causes the destruction of the local periodontal tissue, epidemiological studies have revealed the association between periodontitis and several chronic disorders, such as cardiovascular disease, type 2 diabetes mellitus, rheumatoid arthritis and Alzheimer disease [4,47–50]. Patients with periodontitis exhibit a higher risk of these diseases, and in most cases, the detailed mechanism(s) by which periodontitis exacerbates, or even triggers, systemic pathology have yet to be elucidated. In general, two potential mechanisms have been proposed to link periodontitis to systemic comorbidities: (a) hematogenous dissemination of bacteria associated with periodontitis to extra-oral tissues and (b) spill-over of inflammatory mediators produced in the periodontium into the systemic circulation (Fig. 1) [51]. With regard to the first mechanism, it would be instructive to mention that *P. gingivalis*, a gram-negative anaerobic bacterium that functions as a keystone pathogen in human periodontitis [52], was detected in coronary artery tissue from patients with atherosclerotic cardiovascular disease and femoral artery tissue from patients with atherosclerotic vascular disease-related blockages [53], in pancreatic tissues of patients with diabetes [54] and in Alzheimer's disease (AD) brains [55]. Regarding the potential of inflammatory

cytokine spill-over from the periodontal tissue during routine activities (e.g., tooth brushing, mastication) or after professional dental care (scaling and root planing), the serum levels of IL-1β, IL-6 and MMP-8, which are implicated in atherosclerosis, are significantly higher in patients with periodontitis than in periodontally heathy controls [56,57]. Importantly, several independent studies have reported that resolution of periodontitis by periodontal therapy can improve comorbid disease status or levels of surrogate markers thereof [58–63], thus lending support to the notion of a causal link with periodontitis. Given that complement activation is involved in both the dysbiosis of the periodontal microbiome and the ensuing induction of pro-inflammatory cytokines, it is plausible that treatment of periodontitis with AMY-101 can also mitigate the periodontitis-associated systemic inflammation and pathogen dissemination. Therefore, AMY-101 applied to periodontium locally may be able to improve systemic health condition in patients who suffer from both periodontitis and linked comorbidities (Fig. 1). Of note, a recent study has indicated that complement activation may serve as a mechanism bridging oral dysbiosis with inflammatory sequalae in distant organs. It was shown that *P. gingivalis*-induced complement overactivation in the brain can drive microglial-dependent neuroinflammation and synapse elimination, thereby accelerating cognitive impairment in a mouse model of AD [64].

5. Complement activation in peri-implantitis

Dental implants are widely applied in clinical treatment for missing teeth. The titanium-based fixture enables osseointegration that provides stable support of a prosthesis under functional loads [65]. Although patients who lost their teeth may benefit from treatment by dental implants, a pathological condition, termed peri-implantitis, may adversely affect the oral mucosa and bone around the implants. It is estimated that the rate of peri-implantitis ranges from 7.7% to 21% of dental implants [66–68]. Peri-implantitis is characterized by submucosal microbial dysbiosis and inflammation in the peri-implant connective tissue and progressive, or often rampant, loss of alveolar bone [23]. When the inflammation around a dental implant is confined within the oral mucosa without affecting the alveolar bone, this condition is designated peri-implant mucositis. In analogy to the gingivitis-periodontitis relationship, peri-implant mucositis is considered a precursor to peri-implantitis [69].

There are certain similarities between peri-implantitis and periodontitis regarding clinical features and etiology [70]. For instance, both diseases show radiolucent areas in dental x-ray images, indicative of bone loss, and dental plaque accumulation has a crucial role in aggravating their inflammatory status. However, there are also notable differences between these two oral inflammatory conditions. For example, it was shown that the rate of bone loss progression around implants is faster [71] and thus the size of peri-implantitis lesions is often more extensive than that of periodontitis lesions [23]. Critically, peri-implant defects have strong spatial specificity and most often are well-contained circumferentially around the implant without affecting adjacent teeth [72]. The lesion around the implant body includes larger and more dense areas populated by inflammatory cells, such as plasma cells, macrophages and neutrophils [73]. Moreover, the interaction between the titanium surface of the dental implant and the oral environment results in the release of implant degradation products (titanium particles) into the peri-implant mucosa; the released particles are thought to contribute to the development of inflammatory lesions and are difficult to be effectively removed by implant debridement [72]. Not all implants exhibit biomaterial breakdown and particle release, but certain risk factors exist such as abrasive dental cleaning treatments and inflammatory conditions that can lead to rapid titanium release to the peri-implant plaque and mucosal tissues [74]. These titanium particles function as abiotic immune activation signals in addition to microbial cues in peri-implantitis, by activating complement receptor 3 and scavenger receptor A, and eliciting pro-inflammatory responses and cell death [74–76]. Additionally, the titanium particles induced osteoclastogenesis in vivo [75]. The modification of host immune defenses by persistent abiotic factors is unprecedented from an evolutionary standpoint and can lead to rampant destructive inflammation that requires immunomodulatory therapeutics for management. In corroboration, the peri-implant dysbiotic microbiome resists standard periodontal antibiotic regiments [72]. Thus, it is strenuous to eliminate causes of inflammation once peri-implantitis occurs [77] and it is thus imperative to develop host-modulation therapeutics for peri-implantitis (Fig. 3).

Several reports imply complement involvement in dental implant biology. The proinflammatory complement cleavage products C3a and C5a are generated in vitro when sterile dental implants are incubated with human serum [78]. Similarly, complement components and cleavage products are detected when human buffy coats are cultured with titanium surfaces, whereas surface modification on the implant body by fluoride, which is abundant in the oral environment, affects the concentration of C3 and C4 [79]. Titanium implantation in mice resulted in the triggering of the classical and alternative pathways, upregulation of C3 expression in the tissue surrounding the implant, and activation of osteoclasts [80]. Using an in vitro titanium disk-osteoclast co-culture system, the same study showed that the interaction of osteoclasts with titanium results in the release of C3a, as well as TNF and MMP-9 into the culture supernatants. This proinflammatory response as well as in vitro osteoclast generation was blocked by C3aR blockade, suggesting that C3a may be an effector of Ti-induced osteoclastogenesis [80].

Studies in humans also provide evidence for possible complement involvement in peri-implantitis. In this regard, the GO term: Complement receptor mediated signaling pathway (GO: 0002430) ranked top 10 as significantly enriched GO terms when comparing mRNA isolated from peri-implantitis tissue with that isolated from healthy donors [81]. Our analysis of the dataset GSE 33774 using the GEO2R tool (http://www.ncbi.nlm.nih.gov/geo/geo2r) revealed that the C3 mRNA levels in tissue with peri-implantitis are significantly higher than in both healthy and periodontitis tissue (Fig. 2A) [82]. Fibroblasts harvested from granulation tissue with peri-implantitis expressed the collagen domain of C1q receptor and, upon C1q stimulation, exhibited enhanced expression of inflammatory and chemotactic cytokines (e.g., IL-6, IL-8, MCP-1) and vascular endothelial growth factor VEGF, a key mediator of angiogenesis that is upregulated upon neovascularization [83]. The upregulation of these molecules collectively promotes further infiltration of inflammatory immune cells into the inflamed tissue. Recently, a transcriptome analysis using tissues harvested from healthy and diseased implants within the same individuals identified strong upregulation of the genes encoding for mannan-binding lectin, NLRP3 and IL-1β, all of which are suggestive of complement pathway activation in peri-implant disease [84]. Furthermore, the neutrophil-specific chemokine IL-8 mRNA was more significantly transcribed in disease versus health, which is corroborated by the ex vivo findings of C1q-dependent IL-8 release by peri-implant fibroblasts upon activation [83]. Properdin, which is a positive regulator of complement activation, can be released from several immune cell types [85]. In this regard, our analysis of the dataset GSE 106090 by the GEO2R online tool showed that the properdin mRNA expression is significantly higher in the tissue with peri-implantitis compared to the healthy tissue (Fig. 2B) [81]. Since properdin stabilizes the alternative pathway C3 convertase (C3bBb) increasing its half-life, the upregulation of properdin expression in peri-implantitis tissues may indicate a dysregulated alternative pathway activity that can fuel inflammatory tissue damage through aberrant C3 fragment deposition in adjacent tissues. This could be another mechanism by which complement may be overactivated in peri-implantitis tissue. Additionally, the levels of C-reactive protein (CRP) were increased in peri-implant crevicular fluid (PCF) [86,87] or in serum [88] of peri-implantitis patients. Given that CRP binds to inflamed or apoptotic cells followed by recruitment of C1q and activation of the classical pathway [89], the presence of CRP in peri-implant tissue may contribute to increased complement activation. Matrix

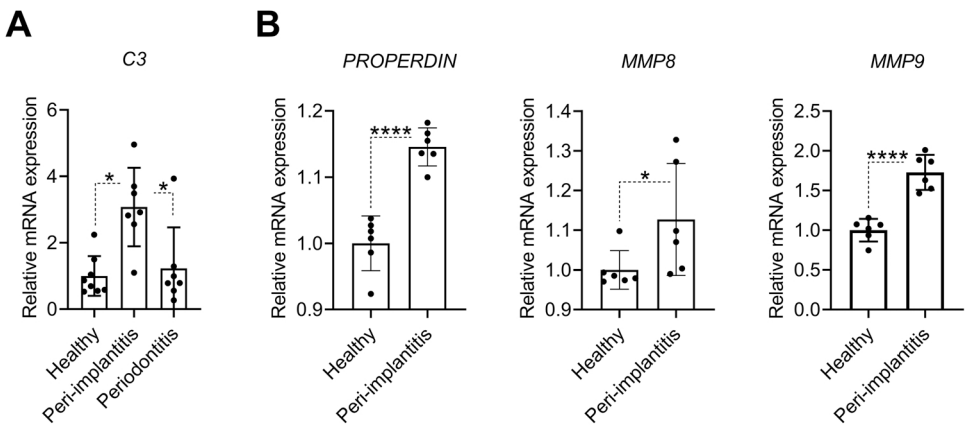

Fig. 2. Upregulation of *C3*, *PROPERDIN*, *MMP8* and *MMP9* in tissue with peri-implantitis. The relative mRNA expression of indicated genes was analyzed using the GEO2R tool in the dataset of GSE 33774 (A) and GSE 106090 (B). Data are means ± SD (A, $n = 8$ for the healthy group and $n = 7$ for the peri-implantitis and the periodontitis group; B, $n = 6$ /group). * $P < 0.05$; **** $P < 0.0001$. One-way ANOVA with Kruskal-Wallis test followed by Dunn's multiple comparison test (A); two-tailed Student's *t*-test (B, *PROPERDIN* and *MMP9*); Mann-Whitney U test (B, *MMP8*).

metalloproteinases MMP-8 and MMP-9 are abundantly expressed in the inflamed gingival tissue and GCF, which causes periodontal tissue destruction [22,90]. Interestingly, the levels of MMP-8 and MMP-9 in PCF and peri-implant soft tissue of patients with peri-implantitis are also increased (Fig. 2B) [91–93] and the former has recently been correlated with marginal bone loss progression [94]. Thus, initial high levels of MMP-8 in PCF could be considered as an indicator of subsequent progression of alveolar bone loss surrounding an implant body [94]. These reports imply that, besides periodontitis, MMPs may have pathological roles also in peri-implantitis. Consistent with this notion, analysis of the GSE33774 microarray dataset using STRING, DisGeNET and Open Targets Platform software revealed that MMP-9 is one of the target genes with a therapeutic potential in peri-implantitis [95]. As mentioned earlier, AMY-101 was shown to reduce the levels of MMP-8 and MMP-9 in the GCF from inflamed periodontal tissue [22]; assuming that AMY-101 could also reduce the levels of MMP-8 and MMP-9 in inflamed peri-implant tissue, this complement-targeted drug may find application as a novel therapeutic for the treatment of peri-implantitis (Fig. 3). Of note, a systematic review revealed that diagnosis or history of periodontitis is associated with increased risk of peri-implantitis [96]. Thus, AMY-101 may be beneficial for patients who suffer from (or are at risk of) both periodontitis and peri-implantitis.

6. Conclusions and outlook

The pathological roles of complement overactivation in periodontitis have been uncovered by several cause-and-effect and mechanistic studies [13,14,22,40]. Therefore, applying the C3 inhibitor AMY-101 to patients who suffer from periodontitis is a rational strategy, especially since C3 is a central hub where different complement initiation pathways converge. AMY-101 exhibited both safety and efficacy to treat periodontitis in NHPs [14,40,41] and, more importantly, the recent phase 2a clinical trial utilizing AMY-101 in patients with periodontal inflammation showed promising results [22]. Although mechanical debridement can often mitigate periodontal inflammation, such a conventional periodontal therapy, represented by scaling and root planing, sometimes provides only temporary improvement followed by disease relapse, particularly in highly susceptible patients [22,97]. Thus, AMY-101 merits further clinical investigation in periodontitis patients. Locally delivered C3 inhibition could lead to an adjunctive therapy which, through host modulation, can enhance the current standard treatment for periodontitis. A multi-center, randomized, double-blind,

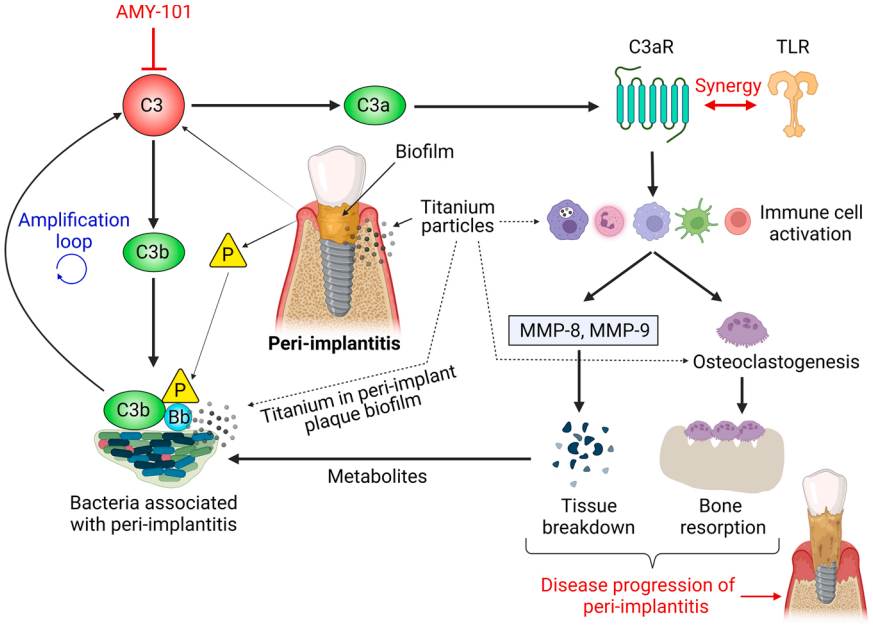

Fig. 3. Complement involvement in peri-implantitis and potential for AMY-101 intervention. The significantly high expression of C3 in the peri-implant tissue and the presence of a dysbiotic microbiota can result in overactivation of complement and thus profound amounts of C3a and C3b in peri-implantitis. C3b binding on the surface of bacteria associated with peri-implantitis forms a C3 convertase, C3bBb. This contributes to shaping an amplification loop producing even more C3a and C3b. The expression of properdin, a positive regulator of complement activation, is also significantly higher in peri-implantitis tissue (vs. healthy tissue), which expedites the amplification loop. These events can potentially result in C3a overproduction. C3a in turn activates C3aR on immune cells which has been shown to synergize with different TLRs. The C3aR-TLR inflammatory synergy can lead to tissue breakdown and bone resorption through induction of MMPs and osteoclastogenesis, respectively, and thereby driving peri-implantitis. As in periodontitis, metabolites released from degraded tissue around implants may exacerbate dysbiosis, which in turn can contribute to the perpetuation of peri-implantitis resulting in a vicious cycle. Titanium particles released by mechanical abrasion can further reinforce this vicious cycle (dotted arrows). By blocking complement activation at the C3 level, locally administered AMY-101 has potential to block inflammatory tissue destruction in peri-implantitis. C3aR, C3a receptor; MMP, matrix metalloproteinase; P, Properdin; TLR, Toll-like receptor.

placebo-controlled, phase 3 clinical trial should provide a clear insight into the potential of AMY-101 as a novel therapeutic for the treatment of periodontal diseases. Since gingivitis is an important risk factor and a pre-requisite for the development of periodontitis [98–100], AMY-101 could also be useful in the treatment of gingivitis.

Epidemiological and clinical intervention studies suggest that periodontitis exacerbates the status of other chronic disorders, presumably through the leakage of bacterial products (e.g. lipopolysaccharide), cytokines and other inflammatory mediators, and dissemination of pathogenic oral bacteria via ulcerated periodontal pockets into the bloodstream [4,101]. Therefore, successful treatment of periodontitis by AMY-101 may additionally reduce the risk of the treated patients for systemic comorbidities.

Preclinical investigations in animal models may provide enhanced insights into the mechanisms by which complement promotes peri-implant dysbiosis and inflammation. Such models of peri-implantitis include both large and medium-size animals (i.e., NHP, canine, and mini pig) [102], whereas recently more convenient peri-implantitis models have been developed in mice [103]. In addition to such preclinical studies, cause-and-effect relationship between complement and peri-implant inflammation can certainly be obtained in clinical intervention studies in human patients (with peri-implant mucositis or peri-implantitis) treated locally with AMY-101. Currently, the disease recurrence rate after non-surgical treatments in peri-implantitis is high [104]. In this regard, even surgical treatments (i.e., resective or regenerative approach) often fail to provide satisfactory outcomes [105,106]. According to several reports discussed in this review, it is plausible that complement pathways are overactivated also in peri-implantitis lesions. Therefore, by blocking C3, the central hub in the complement system, AMY-101 could elicit a broader therapeutic effect attenuating complement activation and peri-implant inflammatory damage in patients with peri-implantitis. In conclusion, preclinical and clinical studies collectively indicate that AMY-101 is a promising novel C3-targeted therapeutic for the treatment of both periodontal and peri-implant inflammatory diseases.

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Conflict of interest

J.D.L. is the founder of Amyndas Pharmaceuticals, which is developing complement inhibitors (including third-generation compstatin analogs such as AMY-101). J.D.L. is inventor of patents or patent applications that describe the use of complement inhibitors for therapeutic purposes, some of which are developed by Amyndas Pharmaceuticals. J. D.L. and G.H. have a joint patent that describes the use of complement inhibitors for therapeutic purposes in periodontitis. J.D.L. is also the inventor of the compstatin technology licensed to Apellis Pharmaceuticals (i.e., 4(1MeW)7W/POT-4/APL-1 and PEGylated derivatives such as APL-2/pegcetacoplan/Empaveli/Aspaveli). The other authors declare no competing interest.

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