C3-targeted therapy in periodontal disease: moving closer to the clinic

George Hajishengallis[1,*], Hatice Hasturk[2,*], Danae A. Apatzidou[3], Georgios N. Belibasakis[4], Nagihan Bostanci[4], Patricia M. Corby[5], Christopher W. Cutler[6], Francesco D'Aiuto[7], Evlambia Hajishengallis[8], Markus Huber-Lang[9], Effie Ioannidou[10], Tetsuhiro Kajikawa[1], Alpdogan Kantarci[2], Jonathan M. Korostoff[11], Georgios A. Kotsakis[12], Tomoki Maekawa[13], Dimitrios C. Mastellos[14], Niki M. Moutsopoulos[15], Srinivas Myneni[16], Richard Nagelberg[17], Bo Nilsson[18], Panos N. Papapanou[19], Evangelos Papathanasiou[20], Jan Potempa[21,22], Antonio Risitano[23], S. Esra Sahingur[11], Atsushi Saito[24], Anton Sculean[25], Andreas Stavropoulos[26,27], Flavia R. Teles[1,28], Maurizio Tonetti[29], Despina Yancopoulou[30], and John D. Lambris[31,*]

[1]University of Pennsylvania, Penn Dental Medicine, Department of Basic and Translational Sciences, Philadelphia, PA, USA, [2]The Forsyth Institute, Cambridge, MA, USA, [3]Aristotle University of Thessaloniki, School of Dentistry, Department of Preventive Dentistry, Periodontology and Implant Biology, Thessaloniki, Greece, [4]Karolinska Institutet, Division of Oral Diseases, Department of Dental Medicine, Stockholm, Sweden, [5]University of Pennsylvania, Penn Dental Medicine, Department of Oral Medicine, Philadelphia, PA, USA, [6]The Dental College of Georgia-Augusta University, Department of Periodontics, Augusta, GA, USA, [7]University College London, Eastman Dental Institute, Department of Periodontology, London, UK, [8]University of Pennsylvania, Penn Dental Medicine, Department of Preventive and Restorative Sciences, Division of Pediatric Dentistry, Philadelphia, PA, USA, [9]University of Ulm, Institute of Clinical and Experimental Trauma immunology, Ulm, Germany, [10]University of Connecticut, School of Dental Medicine, Department of Periodontics, Farmington, CT, USA, , [11]University of Pennsylvania, Penn Dental Medicine, Department of Periodontics, Philadelphia, PA, USA, [12]University of Texas Health Science Center at San Antonio, School of Dentistry, Department of Periodontics, San Antonio, TX, USA, [13]Niigata University, Graduate School of Medical and Dental Sciences, Center for Advanced Oral Science, Gakkocho-dori, Chuo-ku, Niigata, Japan, [14]National Center for Scientific Research 'Demokritos', Division of Biodiagnostic Sciences and Technologies, INRASTES, Athens, Greece, [15]National Institute of Dental and Craniofacial Research, Oral Immunity and Inflammation Unit, Bethesda, MD, USA, [16]Stony Brook University, Department of Periodontology, Stony Brook, NY, USA, [17]Plymouth Meeting Family Dental, East Norriton, PA, USA, [18]Uppsala University Hospital, Clinical Immunology and Transfusion Medicine, Rudbeck Laboratory, Uppsala, Sweden, [19]Columbia University, College of Dental Medicine, Section of Oral, Diagnostic and Rehabilitation Sciences, Division of Periodontics, New York, NY, USA [20]Tufts University, School of Dental Medicine, Department of Periodontology, Boston, MA, USA, [21]Univeristy of Louisville, School of Dentistry, Department of Oral Immunology and Infectious Diseases, Louisville, KY, USA, [22]Jagiellonian University, Faculty of Biochemistry, Biophysics, and Biotechnology, Department of Microbiology, Krakow, Poland, [23]Federico II University of Naples Hematology, Department of Clinical Medicine and Surgery, Hematology, Naples, Italy, [24]Tokyo Dental College, Department of Periodontology, Tokyo, Japan, [25]University of Bern, School of Dental Medicine, Department of Periodontology, Bern, Switzerland, [26]University of Geneva, University Clinics of Dental Medicine, Division of Regenerative Dentistry and Periodontology, Geneva, Switzerland, [27]Malmö University, Faculty of Odontology, Department of Periodontology, Malmö, Sweden, [28]University of Pennsylvania, Center for Innovation and Precision Dentistry, School of Dental Medicine and School of Engineering and Applied Sciences, PA, USA, [29]National Clinical Research Center for Oral Diseases and Shanghai PerioImplant Innovation Center, Shanghai Ninth People Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [30]Amyndas Pharmaceuticals, Glyfada, Greece, [31]University of Pennsylvania, Perelman School of Medicine, Department of Pathology and Laboratory Medicine, Philadelphia, USA.

*Correspondence: George Hajishengallis, geoh@upenn.edu; Hatice Hasturk, HHasturk@forsyth.org; John D. Lambris, lambris@pennmedicine.upenn.edu.

Abstract

Complement plays a key role in immune surveillance and homeostasis. However, when dysregulated or overactivated, complement can switch to a pathological effector, as seen in several inflammatory disorders including periodontal disease, a significant healthcare and socioeconomic burden. Clinical correlative studies and pre-clinical mechanistic investigations collectively demonstrated that complement is hyperactivated in periodontitis and that its interception, at the level of its central component (C3), provides a therapeutic benefit in animal models. The preclinical efficacy of a C3-targeted drug candidate combined with excellent safety and pharmacokinetic profiles supported its use in a randomized, placebo-controlled, double-blind phase 2a clinical study, which showed that C3 inhibition can resolve gingival inflammation in patients with periodontal disease. C3-targeted intervention could represent a novel and transformative host-modulation therapy that merits further investigation in phase 3 clinical trials for the treatment of periodontitis, one of the most common inflammatory diseases of humankind.

Introduction

Periodontitis is a **microbiome**-driven chronic inflammatory disease of the tissues that surround and support the dentition (**Box 1**) [1]. A milder form of periodontal disease, gingivitis, represents inflammation contained within the gingival epithelium and the underlying connective tissue, which commonly precedes the onset of periodontitis. Although gingivitis is reversible, it often persists as chronic inflammation despite daily self-performed control of the dental plaque biofilm and periodic professional care and, therefore, presents a constant risk for periodontitis, which is irreversible in susceptible individuals. Almost half of adults are affected by some form of periodontitis (ranging from mild to severe) with approximately 10% suffering from severe disease [2,3]. The Global Burden of Disease Study has placed severe periodontitis as the 6th most prevalent disease worldwide [4]. If untreated, periodontitis may lead to tooth loss, impaired mastication and esthetics and poorer quality of life [5-9]. Moreover, periodontitis increases the risk of other inflammatory disorders, including cardiovascular disease, rheumatoid arthritis, and Alzheimer's disease [10,11].

Current standard-of-care **periodontal therapy** (see Glossary) aims to remove the pathogenic biofilm through mechanical debridement, often with adjunctive anti-microbial approaches. Current therapy, however, is at times ineffective especially in highly susceptible patients, or does not prevent episodic recurrences; hence, periodontitis remains a formidable public health challenge that results in significant health care expenditure, which is expected to rise given the increase in population longevity [7,9,12-14]. In 2018, the total cost (both direct and indirect due to productivity losses) of periodontal disease in the USA and Europe (32 countries) were estimated at $154.06 billion and €158.64 billion, respectively [15]. Since tissue destruction in periodontitis is mediated primarily by an exaggerated inflammatory response, intentional alteration of the host response (**host modulation**) as an adjunctive therapy may contribute to its management and reduce the risk of associated comorbidities [16]. In contrast, ineffective control of the host's immune response in patients with periodontitis results in continuous progression of the disease over a lifetime. The concept of host-modulation therapy in periodontal disease, however, has remained underdeveloped. Therefore, the potential of

innovative host modulatory strategies to enhance clinical outcomes beyond those achieved via conventional periodontal treatment warrants their development from benchtop to the clinic.

The host inflammatory response and the role of complement

Although historically perceived as a closed system charged with tagging and killing microbes, complement is now appreciated as a key immunological hub for the induction and regulation of diverse immune and inflammatory functions [17]. Complement can shape the overall host immune response via cross-talk interactions with other immune and physiological systems. These include pattern-recognition receptors, such as Toll-like receptors (TLRs) (Figure 1); components of the coagulation system, such as thrombin; and adaptive immune cells, such as T helper 1 (Th1) and Th17 cells [18-20]. Although these properties endow complement with key roles in host immune surveillance and homeostasis, its dysregulation or excessive activation drives pathological inflammation in multiple disorders [21,22].

Besides the classic serum proteins (C1-9), the complement system encompasses some 50 proteins, including pattern-recognition molecules, convertases and other proteases, receptors that interact with different immune mediators, and regulatory proteins in cell-associated or soluble form [23]. The complement cascade can be triggered via distinct mechanisms (*i.e.*, classical, lectin, or alternative pathway) that all converge at the third complement component, C3, which is thus a central target for therapeutic regulation of the downstream immune and inflammatory pathways [24] (Figure 1).

Complement involvement in periodontitis

Pioneering clinical studies by independent groups in the 1970s and 1980s associated periodontitis with increased complement activation in gingival biopsies and the **gingival crevicular fluid** (GCF) obtained from patients relative to healthy control samples [25-29]. An experimental human gingivitis study demonstrated progressive increase of complement cleavage (activation) products that correlated with elevated clinical periodontal inflammation [29]. Consistently, successful periodontal therapy that resolved periodontal inflammation

resulted in reduced C3 activation in the GCF [30]. More recently, the complement activation product C3c was suggested as a potential salivary biomarker for periodontitis [31].

These observational human studies indicated that complement may be involved in the pathogenesis of periodontitis, a notion that was conclusively demonstrated by cause-and-effect studies in preclinical models of periodontitis. Indeed, mice genetically deficient in C3, or in the receptor for the complement anaphylatoxin C3a (C3aR), were protected from developing gingival inflammation and alveolar bone loss, as compared to their wild-type littermate controls [32,33] (Figure 2). Notably, the protective effect of C3 deficiency was confirmed in three distinct disease models, namely, **ligature-induced periodontitis**, *Porphyromonas gingivalis*-induced periodontitis, and naturally-occurring periodontitis [33]. The same study [33] showed that C3 deficiency inhibited the production of cytokines that drive inflammatory bone loss in periodontitis, such as interleukin (IL)-23 and IL-17, which are derived from mainly antigen-presenting cells [34] and Th17 cells [35], respectively. This finding is not only consistent with the ability of complement to cross-talk with and regulate both the innate and adaptive immune response [23], but also implies that complement inhibition alone may have a broader therapeutic effect by blocking downstream inflammation mediated by diverse effectors.

C3-targeted intervention in non-human primate periodontitis

The aforementioned studies in mice suggested that inhibition of complement C3 may provide a therapeutic benefit in the management of periodontitis. The potential efficacy of C3 blockade was addressed using Cp40, a third-generation analog of the compstatin family of cyclic peptidic inhibitors of human and non-human primate (NHP) C3 [24,36]. In human plasma, Cp40 exhibits a half-life of 48 h that exceeds the standards for most peptidic drugs and has a sub-nanomolar affinity for C3. The binding of Cp40 to C3 prevents its cleavage by C3 convertases, thereby blocking the release of the anaphylatoxin C3a and the generation of C3b. The latter inhibitory effect impairs the amplification of the complement response through the alternative pathway of complement activation (Figure 1). Moreover, by inhibiting C3 activation and the assembly of C3b-containing convertases, Cp40 also blocks downstream effector responses, such as the

generation of the anaphylatoxin C5a and the formation of the membrane attack complex [17] (**Figure 1**). Cp40 was clinically developed for human use by Amyndas Pharmaceuticals as 'AMY-101' [24] and is thereafter referred to as such.

The suitability of C3 as a therapeutic target in periodontitis was first tested in NHPs (Figure 2). Specifically, young adult cynomolgus monkeys were subjected to ligature-induced periodontitis under a split-mouth experimental design, *i.e.*, having test and control tooth sites in the same animal [33]. Sites that were locally treated with intragingival injections of AMY-101 (0.1 mg/interdental papilla) showed significantly less alveolar bone loss, as evidenced radiographically, relative to sites treated with a sequence-scrambled control peptide [33]. Moreover, AMY-101 significantly prevented gingival inflammation and clinical attachment loss, as measured by clinical indices. These clinical effects were associated with lower GCF levels of pro-inflammatory and osteoclastogenic cytokines (*e.g.*, IL-17 and RANKL), as well as diminished osteoclastogenesis in bone biopsies [33].

A follow-up study examined the effect of AMY-101 in a therapeutic, rather than preventive, setting. Specifically, the drug was tested for its ability to reverse naturally-occurring periodontitis in aged NHPs [37]. This study involved a 6-week treatment period with injections of AMY-101 in the interdental papillae (0.1 mg/site) and a 6-week follow-up period in the absence of treatment. Regardless of administration frequency (once or three times weekly), AMY-101 significantly reduced clinical indices related to inflammation (Gingival Index and Bleeding on Probing), **periodontal pocket** formation and tissue destruction (Probing Pocket Depth and Clinical Attachment Loss), as well as tooth mobility, as a result of severe periodontitis. Consistent with the clinical findings, AMY-101 also caused a significant reduction of pro-inflammatory mediators in the GCF and osteoclast numbers in bone biopsies. The therapeutic effects of AMY-101 persisted for at least six weeks after treatment completion. GCF samples from the animals of the aforementioned study [37] were subjected to hypothesis-free proteomics characterization [38]. Gene Ontology analysis using Protein Analysis Through Evolutionary Relationships revealed the involvement of the alternative and classical

complement pathways as well as 'leukocyte degranulation' in NHP periodontitis [38]. The latter implied that AMY-101 might also suppress neutrophil exocytosis, a major mechanism of inflammatory tissue destruction [39]. Consistent with this, a later study showed that C3 inhibition by AMY-101 blocks neutrophil extracellular trap release in the plasma of patients with COVID-19 [40].

In yet another study in NHPs, the efficacy of AMY-101 (given at 0.1 mg/site) was tested with decreased frequency of administration, specifically once every two weeks or once every three weeks for a total duration of six weeks [41]. Irrespective of regimen, AMY-101 caused a significant reduction in clinical indices that measure periodontal inflammation (Gingival Index and Bleeding on Probing) as well as pocket formation and tissue destruction (Probing Pocket Depth and Clinical Attachment Loss). Most of these therapeutic effects remained statistically significant for at least 6 weeks after treatment completion, indicating substantial sustainability of its pharmacological effects [41]. Taken together, the NHP studies established that AMY-101 can mediate clinically relevant anti-inflammatory effects in naturally occurring periodontitis.

Safety considerations

The potential of a therapeutic dose of AMY-101 (0.1 mg/site) to cause local irritation after injection was assessed on healthy gingiva of NHPs. AMY-101 and control solution were injected at day 0, 7 and 14. Daily clinical examinations for 28 days revealed no signs of irritation throughout the monitoring period [41]. Although there exists the possibility that intragingival AMY-101 injections may result in systemic exposure, the amount of systemically absorbed AMY-101 is negligible and cannot affect complement functions in circulation or extra-oral tissues. In particular, even if the entire amount of locally administered AMY-101 (1.5 mg needed for treating 15 gingival sites [37]) were injected directly into the circulation, this would result in only 0.2-0.3 mg AMY-101/kg bodyweight in NHPs (or, in case of humans, 0.02–0.03 mg/kg bodyweight). However, a considerably higher systemic AMY-101 dose (1 to 2 mg/kg bodyweight) is required to achieve target-exceeding drug levels [42]. Thus, any amounts of locally injected AMY-101 leaking to the blood circulation should be readily bound by excess C3 (1.0 to 1.5 mg/ml) in the blood. No off-target effects have been observed in animal or human studies with AMY-101, which has also

been used recently for the treatment of severe COVID-19 immunopathology without evidence of any systemic toxicity [40]. Moreover, up to 3 months monitoring of NHPs under systemic exposure to inhibitory levels of AMY-101 (injected subcutaneously at 2 mg/kg bodyweight every 12h for a total of 15 injections) revealed no significant alterations in terms of biochemical, hematological, or immunological parameters in their blood or tissues, as compared to vehicle alone-treated controls [43]. Moreover, the recent FDA approval of the compstatin-based C3 therapeutic empaveli (pegcetacoplan) for the treatment of paroxysmal nocturnal hemoglobinuria validates the clinical safety and efficacy of C3 inhibition by compstatins [44].

A potential concern is whether complement blockade could impair the competency of antimicrobial defenses in the periodontal tissue. However, local inhibition of complement is unlikely to lead to uncontrolled microbial growth in periodontitis. In part, this notion is based on findings that complement is exploited by periodontal pathogens to subvert the host immune response and promote the persistence of a dysbiotic microbial community [45]. Thus, complement activation favors rather than restrains periodontal pathogens, many of which have developed mechanisms to protect themselves from the antimicrobial action of complement [46,47]. Consistently, C3-deficient mice subjected to experimental periodontitis exhibit decreased periodontal bacterial burden compared to wild-type littermate controls [33]. The diminished periodontal inflammation seen in C3 deficiency may also contribute to the reduced microbial burden. In this regard, inflammation is a major ecological factor driving the selective expansion of pathogenic species in periodontitis, presumably by generating a nutritionally favorable environment through accumulation of tissue breakdown products (used as nutrients by the bacteria) [48,49].

Pharmacological inhibition of C3 in human periodontal disease

In 2017, a phase 1 safety trial of AMY-101 was successfully completed in human volunteers (ClinicalTrials.gov Identifier: NCT03316521) [24]. Two years later, AMY-101 received Investigational New Drug approval by the U.S. Food and Drug Administration for the first clinical study to evaluate its efficacy in adults with periodontal inflammation (ClinicalTrials.gov

Identifier: NCT03694444) (Figure 2). A randomized, placebo-controlled, double-blind phase 2a clinical trial was conducted to evaluate the safety and efficacy of AMY-101 in 40 patients with existing periodontal inflammation. AMY-101 was shown to be safe and well-tolerated in all study participants, as evidenced by clinical and laboratory assessments of safety. Corroborating the NHP proof-of-concept studies of C3 inhibition [33,37,41], a once-per-week intragingival injection of AMY-101 for 3 weeks resulted in pronounced and sustainable resolution of gingival inflammation in human subjects. In particular, the clinical efficacy of AMY-101 was reflected by statistically significant reductions in two key periodontal indices measuring gingival inflammation (Gingival Index and Bleeding on Probing) [50]. Consistent with preclinical studies of C3 inhibition in NHP periodontitis [33,37,41], AMY-101 treatment exerted a prolonged anti-inflammatory effect in patients with periodontal inflammation that was evident even 3 months after the initiation of treatment. In light of these promising results, AMY-101 will be further tested in a pivotal Phase 3 study as an adjunctive therapeutic in patients with periodontitis [50].

Conclusion and Outlook

Novel functions attributed to complement in the past two decades have revolutionized our perception of this system from a blood-based antimicrobial effector to a global modulator of immune and inflammatory responses [17]. The multifaceted interactions of complement with other immune cells and physiological systems are reflected in the diversity of inflammatory disorders driven, or exacerbated, by complement dysregulation or overactivation [21,22]. Compelling evidence accumulated over the years indicates that complement is causally linked to periodontitis by inducing destructive inflammation and promoting microbial **dysbiosis** (Figure 1). Accordingly, inhibition of the central complement component C3 by AMY-101 provides a targeted therapeutic benefit without adverse toxicities [50,51]. In the studies conducted thus far, AMY-101 was successfully applied as a stand-alone treatment. However, this C3-targeted drug is intended for use as an adjunctive therapy to the standard periodontal treatment, which by itself is not effective for all patients and thus periodontitis persists as a serious public health and economic burden [7,12-14]. Conceivably, C3-targeted interventions may also be useful in a preventive personalized setting in the case of high-risk individuals (*e.g.,*

patients with diabetes or cigarette smokers). Moreover, since gingivitis is a major risk factor and a necessary precursor of periodontitis [52,53], AMY-101 may also be applicable in the treatment of gingivitis after development of convenient modes of delivery, or as a consumer product.

Whereas basic and translational research in the complement and periodontal disease fields has come a long way, many questions remained to be addressed (see **Outstanding Questions Box**). The documented safety and efficacy of AMY-101 in early-phase clinical trials merits further investigation in future phase 3 clinical trials for the treatment of human periodontitis (**Figure 2**). The phase 3 trial will expand the AMY-101 testing to diverse populations examining its therapeutic effect on both surrogate (*i.e.*, clinical or biological measures of periodontitis) and true periodontal endpoints, such as, maintenance of periodontal health or tooth loss. Ultimately, the goal would be to develop a sustainable intervention, which overcomes the existing limitations of the current standard-of-care treatment of periodontitis.

Glossary

Dysbiosis

An imbalanced, disease-provoking interaction, among the microbial constituents of community and/or between the microbial community and the host immune system. The microbial imbalance results from alterations in the abundance and/or the influence of individual microbial species in disease relative to their abundance or influence in health. Examples of dysbiotic inflammatory disorders are periodontitis and inflammatory bowel disease.

Gingival crevicular fluid (GCF)

Serum exudate originating in the gingival capillaries and flowing into the gingival crevice (or the periodontal pocket). The quantity of this fluid increases with increasing inflammation and contains an abundance of neutrophils as well as locally produced immune and inflammatory mediators, such as complement activation fragments, antimicrobial peptides and cytokines.

Host modulation

A treatment concept that aims to alter the status or function of the host to treat a disease. In an inflammatory disease, host modulation entails efforts to manipulate the immune response in ways that prevent or mitigate tissue damage. In periodontitis in particular, the purpose of host modulation is to disrupt a chronic, self-sustained vicious cycle that links and reinforces microbial dysbiosis and destructive inflammation.

Ligature-induced periodontitis

A model of experimental periodontitis (in both small and large animals) involving the placement of a silk ligature around teeth. Ligature placement generates a subgingival biofilm-retentive milieu leading to microbial dysbiosis, gingival inflammation and bone loss, thus simulating human periodontitis.

Microbiome

A collective term for a diverse microbial community (microbiota) and its combined genetic material and functions within a defined anatomical niche (*e.g.*, the subgingival tooth surface).

Periodontal pocket

The physiological narrow gap between the root of the teeth and the free gingiva is known as subgingival crevice; however, this crevice deepens during progression of periodontitis. This pathologically deepened crevice is designated 'periodontal pocket', a pathognomonic feature of the disease.

Periodontal therapy

The procedure involves mechanical debridement, termed 'scaling and root planing', to remove the microbial biofilm (dental plaque) and calculus (tartar) from the tooth surfaces, including beneath the gingiva, to facilitate inflammation resolution, as well as to smoothen the root surfaces of the teeth in an effort to deter further buildup of dental plaque and calculus.

Acknowledgments

Declaration of interests

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). The other authors declare no competing interest.

Author Contributions

GH, HH and JDL conceived and prepared the original draft and all authors contributed to the writing and/or editing of the final version of the article.

Figure legends

Figure 1: Complement activation and its effects in periodontitis. The complement cascade can be triggered by distinct initiation mechanisms: The classical pathway is initiated by antigen-antibody complex–mediated activation of the C1 complex. The lectin pathway is activated when complexes of mannose-binding lectin (MBL) and MBL-associated serine proteases (MASPs) bind to microbial surfaces. The alternative pathway can be triggered by a 'tick-over' mechanism involving spontaneous hydrolysis of C3, as long as a regulatory mechanism is absent (as is typically the case with microbial surfaces). The so-called 'alternative pathway (AP)-amplification loop' (in which more C3 is cleaved into more C3b which further fuels the loop) amplifies complement activation independently of the initiating mechanism, thereby contributing most of the downstream terminal pathway-mediated effector responses (*i.e.*, C5a, membrane attack complex [MAC]). All three mechanisms of complement activation and amplification converge at C3, which is thus an attractive target of pharmacological interception, *e.g.*, by the cyclic inhibitory peptide AMY-101. If C3 is not blocked, downstream effects include the generation of effectors (C3a and C5a) that promote inflammation and the generation the C5b-C9 MAC with potential antimicrobial but also tissue destructive capacity. Whereas the role of MAC in periodontitis is uncertain, C3a and C5a activate their cognate G-protein-coupled receptors (C3aR and C5aR1), which cross-talk with Toll-like receptors. This cross-talk synergistically activates inflammatory leukocytes, which directly or indirectly mediate destructive periodontal tissue inflammation and bone loss in periodontitis. Complement-mediated inflammation also promotes the dysbiosis of the periodontal microbial community. C3 blockade prevents these downstream effects and thereby offers broader protection against periodontitis.

Figure 2: Milestones to the development of complement C3-targeted intervention in periodontitis. A timeline of key preclinical and clinical studies leading to the development of complement C3-targeted host-modulation, using the peptide-based C3 therapeutic AMY-101 as an adjunctive therapy in periodontal disease (see text for details).

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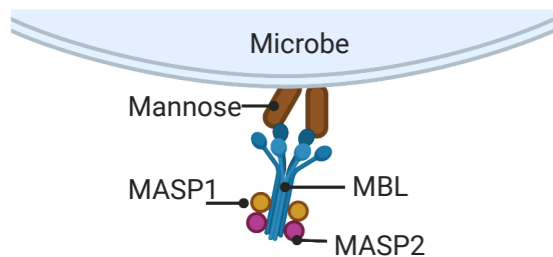
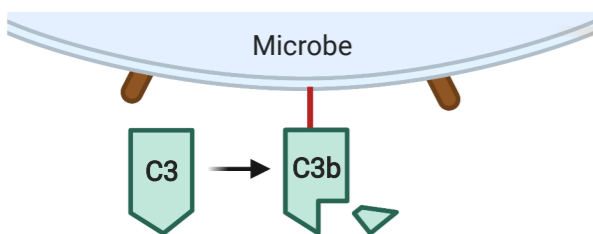**CLASSICAL PATHWAY**

Via Ag-Ab complexes

Microbe

C1

IgG antibody
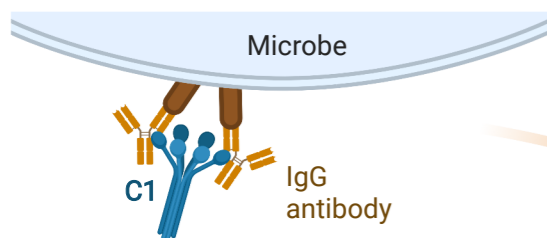
LECTIN PATHWAY

Via MBL-MASP complexes

Microbe

Mannose

MASP1

MBL

MASP2

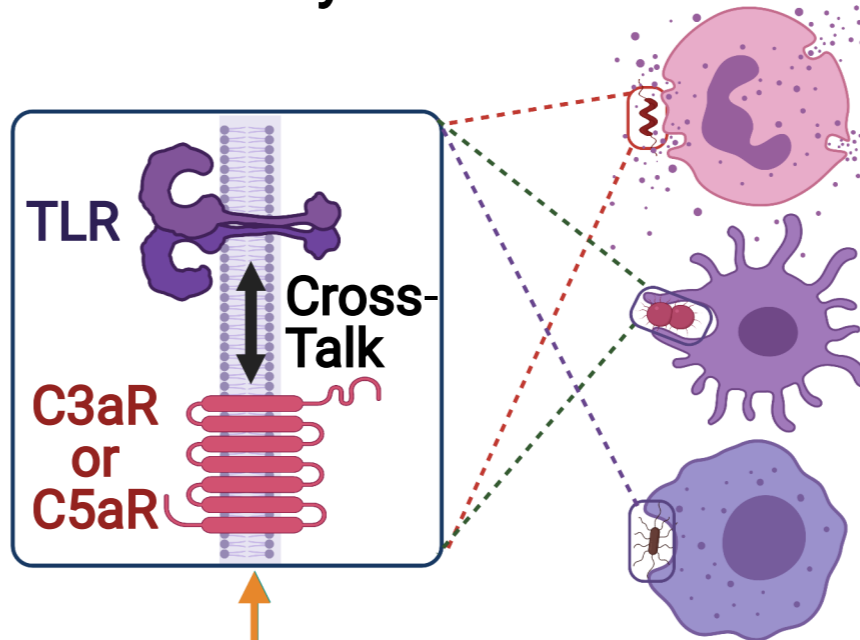**ALTERNATIVE PATHWAY**

Via spontaneous C3 hydrolysis

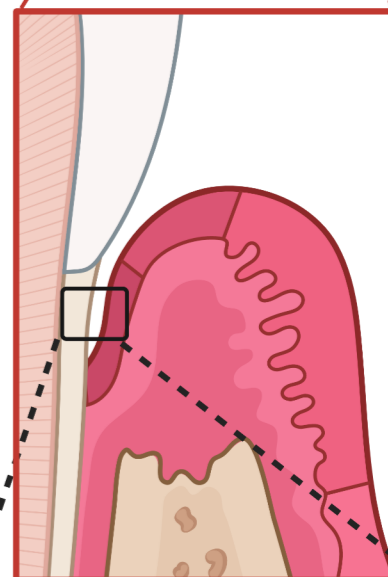Microbe

C3 → C3b

Inflammatory cell activation

TLR

Cross-Talk

C3aR or C5aR

Inflammatory mediators
Degradative enzymes
Reactive oxygen species
Ag presentation & adaptive immunity

C3

AMY-101

STOP

C3a

C3b

C5

C5a

C5b

C6
C7
C8
C9

Self-Amplification Loop

Membrane Attack Complex
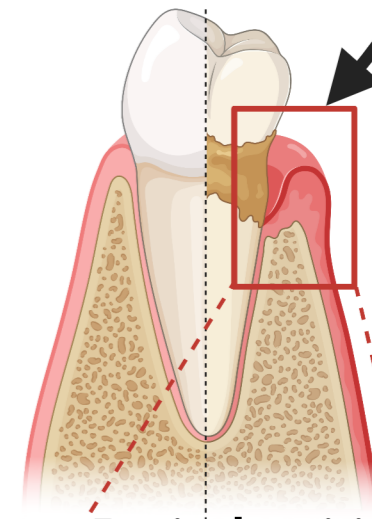
Periodontitis

Dysbiotic microbiome

2011
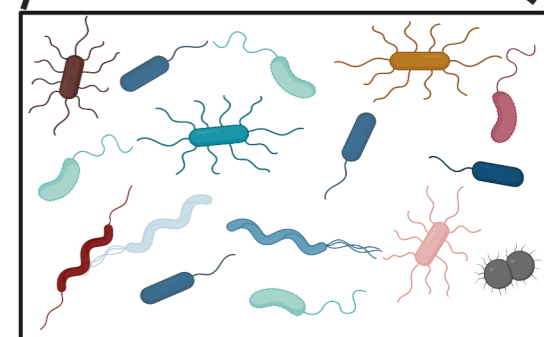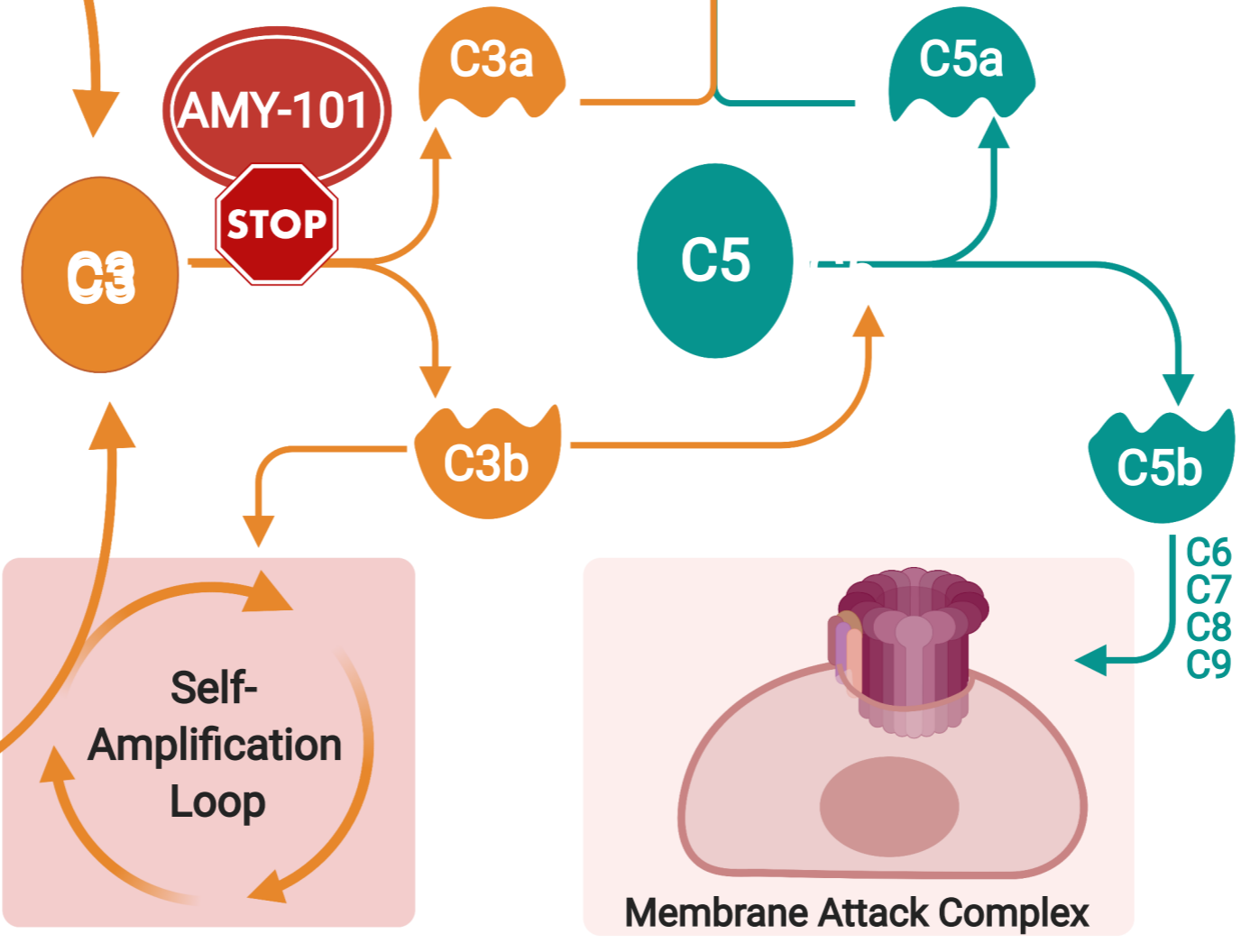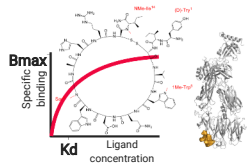
Protection from *P. gingivalis*-induced periodontitis

C3aR$^{-/-}$ mice

2012

2013

2014

3rd-generation compstatin analog with subnanomolar target affinity

Cp40 (AMY-101)

Bmax

Specific binding

Kd

Ligand concentration

C3$^{-/-}$ mice
Protection from experimental periodontitis in three different models

2015

NHPs
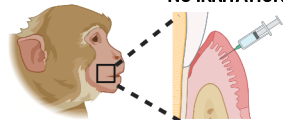AMY-101 treatment; protection from ligature-induced periodontitis

2016

NHPs
AMY-101 treatment; protection from natural periodontitis

2017

NO IRRITATION

AMY-101: Efficacy (even when given once per 3 weeks) & local safety established

NHPs

AMY-101 safety: Successful phase 1 clinical trial

Humans

2018

2019

Humans

U.S. FDA IND approval for phase 2a study to assess AMY-101 efficacy in adults with gingival inflammation

2020

AMY-101 | Placebo

Phase 2a: Resolution of gingival inflammation

Humans

2021

Humans

Projected: Phase 3 clinical trial for the treatment of patients with periodontitis

2022

Box 1. Inflammation in periodontal diseases

The dental plaque biofilm-induced forms of periodontal disease, gingivitis and periodontitis, are prevalent chronic inflammatory conditions that affect distinct and overlapping components of the periodontium, *i.e.*, the tissues that surround and support the teeth, namely the gingiva, cementum, periodontal ligament and alveolar bone [1]. The clinical manifestations of gingivitis include breakdown of the epithelial and connective tissue attachment of the gingiva to the teeth and the deepening of the gingival crevice. In periodontitis, the immunoinflammatory infiltrate is not confined within the gingival epithelium and the underlying connective tissue, but also extends into the deeper compartments of the periodontium. This extensive inflammatory attack leads to the degradation of the cementum, periodontal ligament and the alveolar bone, resulting in tooth mobility and ultimately tooth loss. In both gingivitis and periodontitis, inflammation is initiated by dysbiosis of the local tooth-associated microbiota [49]. Specifically, detrimental inflammation of the periodontal tissues arises when complex microbial communities transition from a commensal to a pathogenic entity that can disrupt tissue homeostasis [49]. The destructive inflammatory response involves the participation and cross-talk of elements of both innate and adaptive immunity, such as, complement, neutrophils, and IL-17-expressing $CD4^+$ T cells (Th17); these interactions culminate in pathologic activation of osteoclasts, which resorb the alveolar bone that supports the teeth [35,37,54]. Periodontal inflammation reinforces and sustains microbial dysbiosis by generating a nutritionally conducive environment, specifically through the accumulation of inflammatory tissue breakdown products (*e.g.*, degraded collagen, a source of amino acids, and heme-containing compounds, a source of iron) [49]. This generates a feed-forward vicious cycle in which destructive inflammation and dysbiosis are reciprocally reinforced and lead to the chronification of periodontal disease.

Outstanding Questions Box

- How long can the host-modulatory effects of C3-targeted inhibition be sustained after treatment of periodontitis patients and what are the mechanisms underlying a sustained therapeutic effect? The answers to these questions may not only provide insights into the homeostatic mechanisms of the periodontium, but will also help develop and optimize clinical treatment regimens.

- Besides regulating the host inflammatory response, does complement blockade in human periodontitis modulate also the dysbiotic microbiome toward a health-compatible configuration? The notion that inflammation is a key ecological factor for dysbiosis is supported by studies in *in vitro* and preclinical models, but clinical evidence is largely lacking.

- Can C3-targeted treatment of patients with periodontitis attenuate systemic inflammatory markers and improve surrogate markers of inflammatory comorbidities? If this can be proven in future studies, it will strengthen the notion that periodontitis is a modifiable risk factor for systemic comorbidities.

- Could local C3 blockade be used as a preventive tool against disease recurrence in patients who have already been treated for periodontitis and are under standard maintenance therapy?

- Is complement involved also in the pathogenesis of other oral inflammatory conditions, such as, peri-implant mucositis and peri-implantitis? If so, could complement-targeted intervention be applied to protect against these hitherto intractable conditions?

Highlights Box

- Complement is a key contributor to immune surveillance and homeostasis; however, when dysregulated or overactivated, complement mediates pathological inflammation.

- Periodontitis is a prevalent chronic inflammatory disease of the tissues that surround and support the teeth and constitutes a significant healthcare and socioeconomic burden.

- Clinical studies have shown that complement is overactivated in periodontitis and that there is a correlation between periodontal inflammation and complement activation.

- Complement involvement in the pathogenesis of periodontitis was conclusively demonstrated in preclinical studies that also identified C3 as a potential target of therapeutic intervention.

- A randomized, placebo-controlled, double-blind phase 2a clinical trial showed that C3-targeted inhibition blocks gingival inflammation in patients with periodontal disease.