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Genetic and Intervention Studies Implicating Complement C3 as a Major Target for the Treatment of Periodontitis

Tomoki Maekawa,* Toshiharu Abe,* Evlambia Hajishengallis,† Kavita B. Hosur,* Robert A. DeAngelis,‡ Daniel Ricklin,‡ John D. Lambris,‡,1 and George Hajishengallis*,1

Chronic periodontitis is induced by a dysbiotic microbiota and leads to inflammatory destruction of tooth-supporting connective tissue and bone. The third component of complement, C3, is a point of convergence of distinct complement activation mechanisms, but its involvement in periodontitis was not previously addressed. We investigated this question using two animal species models, namely, C3-deficient or wild-type mice and nonhuman primates (NHPs) locally treated with a potent C3 inhibitor (the compstatin analog Cp40) or an inactive peptide control. In mice, C3 was required for maximal periodontal inflammation and bone loss, and for the sustenance of the dysbiotic microbiota. The effect of C3 on the microbiota was therefore different from that reported for the C5a receptor, which is required for the initial induction of dysbiosis. C3-dependent bone loss was demonstrated in distinct models, including Porphyromonas gingivalis–induced periodontitis, ligature-induced periodontitis, and aging-associated periodontitis. Importantly, local treatment of NHPs with Cp40 inhibited ligature-induced periodontal inflammation and bone loss, which correlated with lower gingival crevicular fluid levels of proinflammatory mediators (e.g., IL-17 and RANKL) and decreased osteoclastogenesis in bone biopsy specimens, as compared with control treatment. To our knowledge, this is the first time, for any disease, that complement inhibition in NHPs was shown to inhibit inflammatory processes that lead to osteoclastogenesis and bone loss. These data strongly support the feasibility of C3-targeted intervention for the treatment of human periodontitis. The Journal of Immunology, 2014, 192: 6020–6027.

Periodontitis is a prevalent chronic disease (present in >47% of adults in the United States) (1) that features inflammatory destruction of the tooth-supporting tissues (periodontium), such as gingiva and alveolar bone (2). Although the disease is initiated by a dysbiotic microbiota that colonizes subgingival tooth surfaces, it is the host inflammatory response to this microbial challenge that primarily instigates damage upon the periodontium (3). In its severe form that affects 8.5% of adults in the United States (1), periodontitis can conversely affect systemic health by increasing the risk for atherosclerosis, diabetes, rheumatoid arthritis, and adverse pregnancy outcomes (4–7). The graveness of this oral disease and its economic burden (8, 9) underscores the necessity for innovative treatments adjunctive to conventional therapy, which often is not sufficient by itself to control periodontitis (10–13).

Complement is produced locally or systemically and plays an important role in host immune defenses, yet it can also link infection to various local or systemic inflammatory diseases (14, 15). The activation of complement can be triggered via distinct cascade mechanisms (classical, lectin, or alternative), which converge at the third component (C3) and lead to the generation of effectors that mediate diverse functions. These include recruitment and activation of inflammatory cells (via the anaphylatoxins C3a and C5a), microbial opsonization and phagocytosis (via opsonins such as C3b), and direct lysis of susceptible microbes (via the C5b-9 membrane attack complex) (16). Early clinical and histological observations in periodontitis patients have correlated periodontal inflammation and tissue destruction with increased complement activity (17–21). The use of animal models has recently provided mechanistic insights and an emerging model of how complement could mediate periodontitis (22–24). According to this model, complement is a target of immune subversion that leads to the dysbiotic transformation of the microbiota, which, in turn, causes complement-dependent destructive inflammation (23, 25). Specifically, Porphyromonas gingivalis, a Gram-negative anaerobe that is strongly associated with human periodontitis (26), exploits the C5a receptor (C5aR; CD88) to impair innate immunity in ways that promote the overgrowth of the periodontal commensal microbiota, which thereby becomes dysbiotic (22, 27). The commensal microbial community is required for inflammatory bone loss, because P. gingivalis fails to cause periodontitis by itself in germ-free mice despite colonizing this host (22). The notion that commensals can mediate destructive periodontal inflammation is consistent with recent metagenomic studies showing a strong association of hitherto underappreciated commensal bacteria with human periodontitis (28–30).
Although C5aR is crucial for the capacity of *P. gingivalis* to colonize the murine periodontium and cause dysbiosis featuring a marked elevation in the total microbiota counts (22, 24), we reasoned that the ensuing periodontal inflammation could involve additional complement pathways. This idea was substantiated by this study, which has identified critical roles for the central complement component C3. Indeed, whereas C3 was not involved in the induction of dysbiosis, the dysbiotic microbiota required C3 to sustain its presence in high numbers and to cause maximal inflammation and bone loss in mice. Most importantly, we showed that C3 is an appropriate therapeutic target in periodontitis. In this regard, local treatment with an analog of compstatin, a potent C3 inhibitor in humans and nonhuman primates (NHPs) (31), inhibited periodontitis in cynomolgus monkeys. Because NHP periodontitis shares key clinical, microbiological, and immunohistological features with the human disease (32–35), our findings should be highly predictive of drug efficacy in human periodontitis.

### Materials and Methods

#### Bacteria

*P. gingivalis* ATCC 33277 was grown anaerobically from frozen stocks on modified Gifu anaerobic medium–based blood agar plates for 5–6 d at 37°C, followed by anaerobic subculturing for 18–24 h at 37°C in modified Gifu anaerobic medium broth (Nissui Pharmaceutical).

#### C3 inhibitor

The compstatin analog Cp40 (y-I[C(V1MeW)QDW-Sar-AHRC](NMeI)-NH2) and an inactive, sequence-scrambled control peptide (y-I[C-Sar-VDWAH(1MeW)QRC](NMeI)-NH2) were synthesized by Fmoc solid-phase methodology as previously described (36).

#### Animals

All animal procedures were performed according to protocols reviewed and approved by the Institutional Animal Care and Use Committees of the University of Pennsylvania (mice and NHP studies) and of Covance Research Products (Denver, PA, NHP study only), where the NHP work was performed.

**Mice.** C57BL/6 female C3−/− or C5ar−/− mice and corresponding C3+/+ or C5ar+/+ wild-type (WT) littermate controls were obtained from the colonies of Dr. John D. Lembright maintained at The Jackson Laboratory. The C3−/− mice were originally provided by Dr. Rick Weisell (University of Texas) (37). The C5ar−/− mice were originally provided by Dr. Craig Gerday (Harvard Medical School) (38). Mice were maintained in individually ventilated cages, and provided sterile food and water ad libitum under specific pathogen-free conditions. In most experiments, mice were used when they were 8–10 wk old. In experiments of aging, C3−/− and WT mice were reared in parallel and monitored from the age of 5 wk until 9 mo.

**NHPs.** Four adult cynomolgus monkeys (*Macaca fascicularis*) of either sex (3–7 y old, 4–7 kg) were purchased from an approved vendor from stocks that are bred in captivity and were used in the study after a 7-d acclimation period. The animals were socially housed in steel cages elevated off the floor, in a controlled environment with a temperature of 64°F to 84°F and a light/dark cycle of 12:12 h. Environmental enrichment was provided through daily handling by animal care technicians, environmental enrichment items, visual contact with other study animals, and appropriate background music in the animal facility. Each animal was offered a measured amount of an approved feed mixture. Fresh, potable drinking water was available to the animals ad libitum. Clinical periodontal examinations, dental X-rays, collection of gingival crevicular fluid (GCF), and periodontal tissue biopsies were performed in a manner similar to a human clinical study. The animals were not euthanized at the completion of the study.

### P. gingivalis colonization and induction of periodontitis in mice

Periodontal inflammation and bone loss were induced in specific pathogen-free mice by oral inoculation with *P. gingivalis*, essentially as originally described by Baker et al. (39). In brief, by means of a ball-ended feeding needle, mice were orally inoculated five times at 2-d intervals with 10^7 CFU of *P. gingivalis* suspended in 2% carboxymethylcellulose vehicle. Sham-inoculated controls received vehicle alone. The mice were euthanized 42 d after the last oral inoculation. Periodontal bone loss was assessed morphometrically in defleshed maxillae using a dissecting microscope (×40) fitted with a video image marker measurement system (Nikon Instruments). Specifically, the distance from the cement–enamel junction (CEJ) to the alveolar bone crest (ABC) was measured on 14 predetermined points on the buccal surfaces of the maxillary molars (39). The 14-site total CEJ-ABC distance for each mouse was subtracted from the difference CEJ-ABC distance of sham-injected mice to calculate bone loss. The results were expressed in millimeters, and negative values indicated bone loss relative to sham controls.

The levels of *P. gingivalis* colonization and the number of total bacteria in the periodontal tissue were determined using quantitative real-time PCR (qPCR) of the *ISP*/*ISP* gene (*P. gingivalis*) and the 16S rRNA gene (total oral bacteria) (22, 40). *ISP*/*ISP* was selected to index the sensitivity of *P. gingivalis* detection, because this gene is present in 31 copies in the genome of *P. gingivalis* ATCC 33277 (the gene copy numbers were therefore divided by 31 to obtain genome equivalents). For this purpose, genomic DNA was isolated from maxillary periodontal tissue (including both soft and hard tissue, that is, teeth and immediately surrounding bone) using the DNeasy kit (Qiagen) and was quantified by spectrophotometry at 260 and 280 nm. qPCR was performed using the ABI 7500 Fast System (Applied Biosystems). TaqMan probes, sense primers, and antisense primers used were purchased from Applied Biosystems. The primer sets used for the quantification of *P. gingivalis* and total bacteria were published previously (40).

### Ligature-induced periodontitis in mice

The placement of ligatures accelerates bacteria-mediated inflammation and bone loss (41). To this end, a 5–0 silk ligature was tied around the maxillary left 42. The contralateral molar tooth in each mouse was left untreated to serve as baseline control in the bone-loss measurements. The mice were euthanized 5 d after placement of the ligatures, and defleshed maxillae were used for CEJ-ABC distance measurements using a morphometric method (see earlier). Bone height measurements were performed on the ligated second molar (three sites corresponding to mesiopalatal cusp, palatal groove, and distopalatal cusp) and the adjacent regions (sites corresponding to distopalatal groove and distal cusp of the first molar, and palatal cusp of the third molar) (42). The six-site total CEJ-ABC distance for the ligated side of each mouse was subtracted from the six-site total CEJ-ABC distance of the contralateral unligated side to calculate bone loss. The results were presented in millimeters, and negative values indicated bone loss relative to the baseline (unligated control).

### Clinical examinations, periodontitis, and sample collection in NHPs

Four adult cynomolgus monkeys (see earlier for details) were used for local (intrargival) administration of Cp40, the most potent compstatin analog to date, or an inactive peptide control (31, 36). All treatments and clinical examinations were performed on previously anesthetized animals. Experimental periodontitis was induced by tying *P. gingivalis*-soaked ligatures (size 2 silk) around posterior teeth (second premolars and first molars). Ligatures were placed on both halves of the mouth for a split-mouth experimental design to reduce the number of animals required; that is, one side was treated with active drug (Cp40) and the other with inactive control. Cynomolgus monkeys naturally harbor *P. gingivalis* at variable levels (43), and the use of *P. gingivalis*-soaked ligatures aimed to even out potential differences for more uniform results.

Clinical examinations and diagnosis were performed according to the criteria of the American Academy of Periodontology (44). Examinations using a periodontal probe were performed at baseline and throughout the study (weeks 1, 2, 4, 6) to monitor the progression of the disease and the effects of Cp40. The examinations included determination of clinical attachment loss (CAL), probing pocket depth (PPD), bleeding on probing, gingival index, and tooth mobility index (Mob) at the mesiobuccal, midbuccal, and distobuccal, and mesial and distal aspects of each of the premolar and molar maxillary teeth. At the same sessions that clinical examinations were performed, GCF was collected using PerioPaper strips (Oraflow) placed between the gums and the teeth, specifically in the mesiobuccal sulcus of each ligated posterior tooth, for 30 s. At baseline and at the completion of the study (6 wk), standardized bitewing dental X-ray images were taken using high-speed dental X-ray films to evaluate bone loss. The bone height were determined from the X-ray images using the NewTom System software. Specifically, CEJ-ABC distances were measured at six points (first premolar, distal; second premolar, mesial and distal; first molar, mesial and distal; second molar, mesialal), and the data shown in...
FIGURE 1. C3 deficiency protects against P. gingivalis–induced inflammatory periodontal bone loss. C3+/+ (WT) or C3−/− mice were orally inoculated with P. gingivalis or vehicle only (sham) and 42 d postinoculation were assessed for periodontal bone loss (A) and for mRNA (B) and protein (C) expression of the indicated cytokines in the gingiva. The mRNA expression levels were normalized against GAPDH mRNA and expressed as fold induction relative to the transcript levels of sham-infected WT mice, which were assigned an average value of 1. Data are means ± SD (n = 5 mice/group). *p < 0.01 compared with sham-infected WT, **p < 0.01 between P. gingivalis–infected C3−/− and P. gingivalis–infected WT (ANOVA; main p < 0.001 in each experiment analyzed).
C5ar ligature-induced periodontal bone loss as compared with WT C3 inflammatory periodontal bone loss (50, 51). To this end, we associated periodontal bone loss with Fig. 1, these data suggest that C3 is not required for bone loss. Although dysbiotic microbiota and the induction of maximal inflammatory microbiota counts, but it is crucial for long-term sustenance of the periodontium of WT or complement-deficient mice. WT, P. gingivalis colonization and its effects on the microbiota in at least three independent murine models, ranging from in vivo experiments in humans and NHPs (31). To determine the suitability of C3 as a therapeutic target in periodontitis, we designed ligature-induced periodontitis studies in cynomolgus monkeys and tested the efficacy of Cp40, the most potent analog of compstatin reported thus far (31). To this end, P. gingivalis–soaked silk ligatures were placed around maxillary posterior teeth (second premolar and first molar) on both halves of the mouth for a split-mouth experimental design; that is, one side was locally injected in the gingiva with active drug (Cp40) and the other side with inactive peptide analog (control). Thus, each animal served as its own control. An initial periodontitis study with a 6-wk duration was conducted using two animals, in which treatments started 3 d after placing the ligatures and continued three times weekly throughout the study. In both animals, treatments with Cp40 resulted in decreased clinical inflammation parameters (Supplemental Fig. 1) correlating with lower levels of proinflammatory cytokines in the GCF and decreased numbers of osteoclasts in bone biopsy specimens (Supplemental Fig. 2).

To confirm and expand on these pilot findings, we performed a second NHP study, in which ligatures were placed around the mandibular posterior teeth (i.e., in the lower jaw) of the same two animals and in two additional animals. The inclusion of four animals in the second study allowed the possibility for statistical analysis. Similar to the original study, treatment with Cp40 caused a reduction in clinical indices that measure periodontal inflammation and tissue destruction (Fig. 4). The protective effects of Cp40 reached statistical significance (p < 0.05) for CAL (Fig. 4A) and gingival index (Fig. 4B). Although such significance was not reached for bleeding on probing (Fig. 4C) and mobility index (Mob; Fig. 4D) after 6 wk of treatment, there were obvious differences between the Cp40- and control-treated sides. Most importantly, radiographic analysis showed that Cp40 caused a significant inhibition of bone loss (Fig. 5A–C). Specifically, whereas Cp40-treated and control-treated sites had similar bone heights (CEJ-ABC distances) at baseline (Fig. 5A), all Cp40-treated sites had lower bone heights than the corresponding contralateral control sites by the end of the 6-wk experimental period.

**FIGURE 2.** P. gingivalis colonization and its effects on the microbiota in the periodontium of WT or complement-deficient mice. WT, C3−/−, or C5ar−/− mice were orally inoculated with P. gingivalis or vehicle only (Sham) and were euthanized 7 (A) or 42 d (B) later. The numbers of P. gingivalis and of total bacteria in the periodontal tissue were determined using qPCR of the ISPg1 gene (P. gingivalis) or the 16S rRNA gene (total bacteria). Data are means ± SD (n = 5–6 mice/group). The WT group included three C3+/+ and three C5ar+/+ mice that yielded similar results and were grouped together. *p < 0.01 between indicated groups (unpaired t test).

**FIGURE 3.** C3−/− mice are protected against ligature-induced or aging-associated periodontal bone loss. (A) Periodontal bone loss was induced in C3+/+ (WT) or C3−/− mice by placing a silk ligature around the maxillary left second molar, whereas the contralateral tooth was not ligated to serve as baseline control. Negative values indicate bone loss relative to the unligated contralateral side. (B) Time course of naturally occurring bone loss in aging WT mice as compared with age-matched C3−/− mice; negative values indicate bone loss relative to bone measurements in 5-wk-old WT mice. Data are means ± SD [(A), n = 5; (B), n = 5–6 mice/group]. *p < 0.01 between indicated groups (A) or compared with age-matched WT control (B) (unpaired t test).

**Locally targeted inhibition of C3 protects against periodontitis in NHPs**

The peptide compstatin and its newer analogs block the activation of C3 by convertases and show exclusive specificity for C3 of humans and NHPs (31). To determine the suitability of C3 as a therapeutic target in periodontitis, we designed ligature-induced periodontitis studies in cynomolgus monkeys and tested the efficacy of Cp40, the most potent analog of compstatin reported thus far (31). To this end, P. gingivalis–soaked silk ligatures were placed around maxillary posterior teeth (second premolar and first molar) on both halves of the mouth for a split-mouth experimental design; that is, one side was locally injected in the gingiva with active drug (Cp40) and the other side with inactive peptide analog (control). Thus, each animal served as its own control. An initial periodontitis study with a 6-wk duration was conducted using two animals, in which treatments started 3 d after placing the ligatures and continued three times weekly throughout the study. In both animals, treatments with Cp40 resulted in decreased clinical inflammation parameters (Supplemental Fig. 1) correlating with lower levels of proinflammatory cytokines in the GCF and decreased numbers of osteoclasts in bone biopsy specimens (Supplemental Fig. 2).

To confirm and expand on these pilot findings, we performed a second NHP study, in which ligatures were placed around the mandibular posterior teeth (i.e., in the lower jaw) of the same two animals and in two additional animals. The inclusion of four animals in the second study allowed the possibility for statistical analysis. Similar to the original study, treatment with Cp40 caused a reduction in clinical indices that measure periodontal inflammation and tissue destruction (Fig. 4). The protective effects of Cp40 reached statistical significance (p < 0.05) for CAL (Fig. 4A) and gingival index (Fig. 4B). Although such significance was not reached for bleeding on probing (Fig. 4C) and mobility index (Mob; Fig. 4D) after 6 wk of treatment, there were obvious differences between the Cp40- and control-treated sides. Most importantly, radiographic analysis showed that Cp40 caused a significant inhibition of bone loss (Fig. 5A–C). Specifically, whereas Cp40-treated and control-treated sites had similar bone heights (CEJ-ABC distances) at baseline (Fig. 5A), all Cp40-treated sites had lower bone heights than the corresponding contralateral control sites by the end of the 6-wk experimental period.
bone loss was calculated as bone height at baseline minus bone height at
and at wk 6, respectively. For each pair of control and Cp40 treatments,

Methods

Materials and software and standardized X-ray images (taken at baseline and at wk 6).

heights (CEJ-ABC distance) were measured using Nikon Imaging System
treated as described in the legend to Fig. 4, and their mandibular bone

FIGURE 4. Cp40 decreases inflammatory clinical parameters of NHP periodontitis. Starting 3 d after initiation of ligature-induced periodontitis, Cp40 or control were injected locally into the mandibular interdental papillae from the first premolar to the second molar, three times weekly, in opposites sides of the mouth (split-mouth design). The animals were clinically examined at the indicated time points, and the effects of Cp40 on the following inflammatory clinical parameters were recorded: (A) clinical attachment loss (CAL), (B) gingival index (GI), (C) bleeding on probing (BOP), and (D) mobility index (MI). At the beginning of the study, the gingival margins in all animals were at the CEJ, and thus CAL readings equaled PPD; hence PPD is not shown. Data are means ± SD (n = 4 monkeys). *p < 0.05, **p < 0.01 compared with time-matched control (paired t test).

FIGURE 5. Inhibition of periodontal bone loss and osteoclastogenesis after treatment of NHP periodontitis with Cp40. (A–C) Four monkeys were treated as described in the legend to Fig. 4, and their mandibular bone heights (CEJ-ABC distance) were measured using Nikon Imaging System software and standardized X-ray images (taken at baseline and at wk 6). Measurements were made at six points (specified in Materials and Methods), and the data in (A) and (B) reflect the six-site total at baseline and at wk 6, respectively. For each pair of control and Cp40 treatments, bone loss was calculated as bone height at baseline minus bone height at 6 wk (C); the difference between control and Cp40 treatments was significant (p < 0.05). (D) TRAP+ multinucleated cells (osteoclasts) were enumerated in nine serial sections for each bone biopsy specimen taken between the second premolar and first molar, from control or Cp40-treated sites of all animals. The numbers of osteoclasts were averaged for each control or Cp40-treated specimen, and the data are shown as means ± SD (n = 4 monkeys). *p < 0.01 compared with control (paired t test).

FIGURE 5C). The inhibition of bone loss by Cp40 correlated
with significantly decreased osteoclast numbers in bone biopsy samples (Fig. 5D).

Multicytokine analysis of the GCF revealed that Cp40 treatment resulted in significantly lower levels of most proinflammatory cytokines tested, including TNF, IL-1β, IL-17A, and RANKL, a key osteoclastogenic factor produced by activated lymphocytes and stromal/osteoblastic cells (52) (p < 0.05; Fig. 6). In contrast, the GCF levels of OPG, a natural inhibitor of RANKL (52), were maintained at higher levels in Cp40-treated sites than in control sites during the course of the study (Fig. 6I). The Cp40-mediated inhibition of IL-17A, a major bone-resorptive cytokine (52), and the differential effects of Cp40 on RANKL and OPG production were confirmed by fluorescent immunohistochemistry of periodontal biopsy specimens (Fig. 7A). RANKL expression was linked to osteoclastogenesis because it was detected in regions positive also for cathepsin K (Fig. 7B), a protease involved in bone resorption expressed predominantly in osteoclasts (53); moreover, TRAP+ cells were detected in adjacent serial sections (Fig. 7B, bottom). This study marks the first time, to our knowledge, that complement is implicated in inflammatory bone loss in NHPs and provides a promising target for therapeutic intervention.

Discussion

Our findings from distinct mouse models implicated C3 in periodontal inflammation and bone loss. A recent systematic study, which compared transcriptional responses with systemic inflammatory challenges in mice and humans, suggested that mice might not be reliable models to study human inflammatory diseases (54). It was therefore important to confirm the role of C3 in periodontitis using NHP, the closest model to human periodontitis (33). Indeed, the immune system, periodontal anatomy, and clinical features of periodontitis are similar between humans and cynomolgus monkeys (32–35). The capacity of complement C3 or C5aR signaling to potentiate human and NHP C3 (31), to block periodontal inflammation and bone loss in cynomolgus monkeys provides unequivocal support for the appropriateness of C3 as a treatment target for human periodontitis.

The host protective mechanism(s) associated with C3 inhibition in periodontitis may not be restricted to mere suppression of the proinflammatory activities of the complement cascade itself, because complement effector pathways (e.g., C3a or C5aR signaling) cross talk with and amplify TLR-mediated inflammatory responses in both systemic and mucosal settings (55, 56) including the periodontal tissue (24). Complement inhibition, therefore, can also attenuate inflammation initiated by TLR activation. Interestingly, TLR activation is not exclusively triggered by microbial ligands. For instance, TLR2 and TLR4 can also be activated by endogenous molecules, which are released after inflammatory tissue destruction and act as danger signals (e.g., biglycan, hyaluronan fragments, and heparan sulfate fragments) (57, 58). Complement may thus be involved in the amplification of inflammation by released endogenous TLR ligands in the course of periodontitis and, consequently, may contribute to the progression of the disease. The involvement of C5aR in periodontal dysbiosis (59) suggests a role for complement also in the initiating stages of periodontitis. We have now additionally shown that complement, specifically C3, is also required for the sustenance of dysbiosis. Therefore, the therapeutic targeting of complement is likely to interfere with multiple stages in the development of periodontal disease.

Complement and TLRs participate in the regulation of IL-17A production by both innate and adaptive immune cells (24, 60–64). Although complement generally exerts complex effects on IL-17A expression that include both positive and negative regulation, we
have previously shown that complement augments IL-17A production in the murine periodontal tissue in synergy with TLRs (24). Consistently, periodontal IL-17A production was potently inhibited by C3 deficiency in mice or by Cp40 treatment in NHP in our studies. IL-17A can play a crucial role in bone immunopathology by inducing the expression of matrix metalloproteinases and RANKL, thereby potentially contributing (together with other cytokines such as TNF and IL-1β) to the destruction of both connective tissue and the underlying alveolar bone in periodontitis (3, 52). The inhibition of RANKL expression by Cp40 in NHP periodontitis may, in part, be a consequence of the ability of Cp40 to inhibit IL-17A. The Cp40-mediated inhibition of RANKL expression may, in turn, be responsible for the observed inhibition of osteoclastogenesis and bone loss. However, we cannot exclude the possibility that complement inhibition by Cp40 may additionally have direct effects on osteoclasts, which were recently shown to express complement receptors (65). The RANKL/OPG ratio in the GCF increases with escalating inflammatory activity and is thought to be a useful biomarker for human periodontitis (66). Our findings support this notion because the inhibition of NHP periodontitis correlated with decreased RANKL but increased OPG levels in Cp40-treated sites as compared with control-treated sites. 

P. gingivalis and other immune-subversive periodontal bacteria, including Tannerella forsythia, Treponema denticola, and Prevotella intermedia, interact with complement in complex ways that include both inhibitory and stimulatory effects (67–71). This seemingly conflicting microbial behavior could be explained by the dynamics of the survival tactics of periodontal bacteria: on the one hand, periodontal bacteria need to evade immune elimination, whereas, on the other hand, the bacteria have to proactively stimulate inflammation and the flow of GCF to obtain essential nutrients (e.g., tissue breakdown peptides and hemin-containing compounds) (12, 23). The dependence of periodontitis-associated bacteria on inflammation provides an explanation as to why dysbiosis could not be sustained in C3−/− mice, which had significantly lower periodontal inflammation than WT mice. This novel finding is consistent with the emerging notion that anti-inflammatory treatments in periodontitis can also exert antimicrobial effects (12, 51). Our findings also highlight fundamental differences between C3 and C5aR in terms of their impact on the
periodontal microbiota, at least in mice: whereas C5aR is crucial for P. gingivalis colonization and its capacity to boost the numbers of the commensal microbiota, C3 is not required for the initial rise of the pathobiotic microbiota but contributes to its sustenance. As alluded to earlier, inflammation exerts a significant impact on the periodontal microbiota. Indeed, under conditions of disrupted homeostasis, there is a blooming of inflammatory bacteria that act as pathobiotics and exacerbate inflammatory tissue destruction in periodontitis (72, 73). These recent advancements provide experimental support for the ecologic plaque hypothesis formulated >10 y ago (74). This hypothesis predicted that “pathogens” are components of the commensal microbiota but at levels too low to cause disease; changes in ecologic conditions favor the overgrowth of these organisms beyond a threshold sufficient to cause or exacerbate disease (74). In brief, periodontitis is not a “classic” infection in which the causative agents are derived from an exogenous source, but rather an ecologic disease in which host modulation may be a highly appropriate way of therapeutic intervention.

There is currently an unmet need for efficacious and safe therapeutics for chronic diseases such as periodontitis, which is often unresponsive to conventional treatment (scaling and root planing) (10–13). Comstatin-derived compounds have been successfully tested in terms of safety and efficacy in several other disease models, including treatment of sepsis and hemodialysis-associated inflammation, and a compstatin analog is currently in clinical development for the treatment of age-related macular degeneration (reviewed in Ref. 31). Cp40 itself has recently shown high efficacy in models of paroxysmal nocturnal hemoglobinuria and good tolerability after systemic application in NHP (75). This study suggests another potentially promising clinical application for compstatin-derived analogs. Indeed, the capacity of Cp40 to block experimental periodontitis in NHP suggests that it is likely to be translated to the treatment of human periodontitis as a locally applied (hence even safer than systemic) therapeutic.

Disclosures

G.H. and J.D.L. have a joint patent application that describes the use of complement inhibitors for therapeutic purposes in periodontitis. D.R. and J.D.L. are the inventors of patents and/or patent applications that describe the use of complement inhibitors for therapeutic purposes. J.D.L. is the founder of Amyndas Pharmaceuticals, which is developing complement inhibitors for therapeutic purposes in periodontitis. D.R. and J.D.L. have a joint patent application that describes the use of complement inhibitors for therapeutic purposes in periodontitis. D.R. and J.D.L. are the inventors of patents and/or patent applications that describe the use of complement inhibitors for therapeutic purposes in periodontitis. D.R. and J.D.L. have a joint patent application that describes the use of complement inhibitors for therapeutic purposes in periodontitis.

References


Supplemental figure 1. Inhibition of inflammatory clinical parameters following treatment of NHP periodontitis with Cp40. Starting 3 days after initiation of ligature-induced periodontitis, Cp40 (500 µg) was injected locally into the maxillary interdental papillae from the first premolar to the second molar, in two animals, three times weekly. An inactive peptide control of Cp40 was injected into the contralateral side of the mouth in the same two animals (split-mouth design). Shown are the effects of Cp40 on the indicated inflammatory clinical parameters. At the beginning of the study, the gingival margins in all animals were at the cement-enamel junction, and thus CAL readings equaled probing pocket depth (PPD); hence, PPD is not shown.
Supplemental figure 2. Cp40 inhibits proinflammatory cytokine production and osteoclastogenesis in NHP periodontitis. At the same time points that clinical examinations were performed (Supplemental Fig. 1), gingival crevicular fluid (GCF) was collected from the same monkeys analyzed in Supplemental Fig. 1, using PerioPaper strips to assay the indicated cytokines. Total cytokine content in the eluted GCF samples was measured using Milliplex xMap kits on a Bio-Plex system. In panel G, TRAP-positive multinucleated cells (osteoclasts) were enumerated in nine serial sections for each bone biopsy specimen taken between the second premolar and first molar, from control- or Cp40-treated sites of the two animals.