Chapter 13
Complement-Targeted Therapeutics in Periodontitis

George Hajishengallis and John D. Lambris

Abstract Periodontitis is a prevalent oral chronic inflammatory disease which, in severe forms, may exert a major impact on systemic health. Clinical and histological observations, as well as experimental animal studies, suggest involvement of the complement system in periodontitis. However, the precise roles of the various complement components and pathways in periodontitis have only recently started to be elucidated. In this chapter, we review recent progress in the field and discuss the potential of complement-targeted therapeutics in the treatment of periodontitis.

13.1 Introduction

Periodontal disease is a prevalent chronic condition that causes inflammatory destruction of the tooth-supporting tissues (Pihlstrom et al. 2005). Severe periodontitis exerts a systemic impact on health, and the patients run increased risk for systemic diseases, such as atherosclerosis, diabetes, aspiration pneumonia, averse pregnancy outcomes, and perhaps rheumatoid arthritis (Tonetti et al. 2007; de Pablo et al. 2009; Kebschull et al. 2010; Pihlstrom et al. 2005; Scannapieco et al. 2010; Genco and Van Dyke 2010). Treated periodontal patients often develop recurrent disease for reasons that are not clear, thus necessitating better understanding of the underlying immunopathology (Armitage 2002; Hajishengallis 2009b). The annual cost of periodontal therapy in the USA exceeds $14 billion (Brown et al. 2002), and the suspected association of periodontitis with systemic conditions underscores the importance of implementing new and effective treatment options.

Although a group of tooth-associated subgingival anaerobic bacteria is strongly associated with periodontitis (Socransky et al. 1998), it is the host inflammatory response to uncontrolled bacterial challenge, rather than direct bacterial toxic effects, that primarily mediates periodontal tissue destruction (Gaffen and Hajishengallis 2008; Graves 2008). In this context, periodontal health represents a dynamic state where proinflammatory and antimicrobial activities are optimally regulated to prevent unwarranted host reactions (Gaffen and Hajishengallis 2008). This homeostatic balance may be disrupted, however, either by genetic immunoregulatory defects or by pathogens that subvert the host
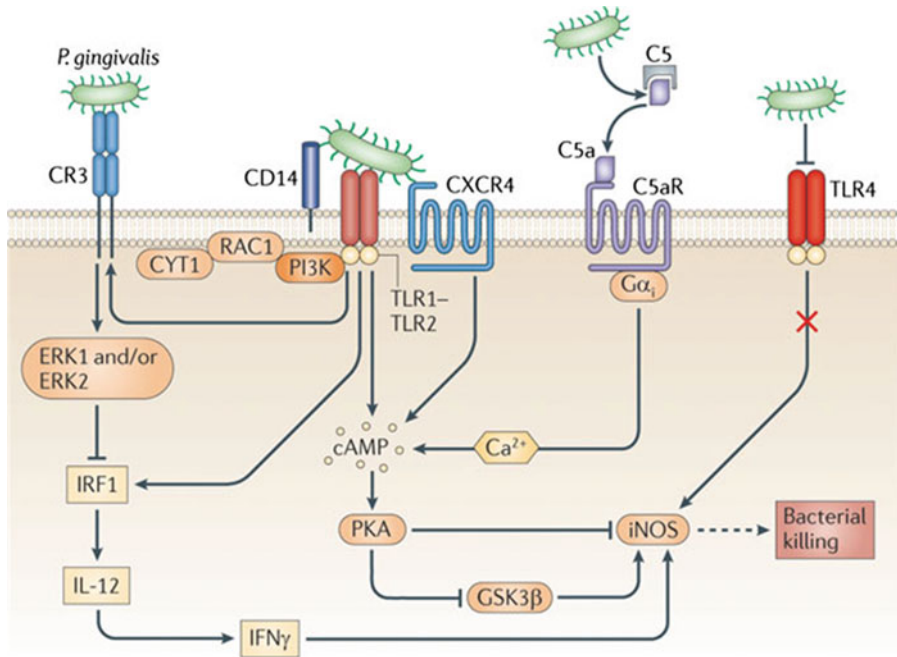
G. Hajishengallis (✉)
Department of Microbiology, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
e-mail: geoh@upenn.edu

J.D. Lambris
Department of Pathology and Laboratory Medicine, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Fig. 13.1 *Exploitation of C5aR and other innate immune receptors by P. gingivalis to undermine host immunity. P. gingivalis* has surface structures that interact with Toll-like receptor (TLR)-2 (specifically with the CD14–TLR2–TLR1 signaling complex) and with TLR4. The activation of TLR4, however, is blocked by the bacterium's atypical lipopolysaccharide which acts as an antagonist; therefore, TLR4 is unlikely to induce protective responses. The TLR2 response is proactively modified through crosstalk with other receptors that are under *P. gingivalis* control. *P. gingivalis* regulates C5a receptor (C5aR) by virtue of Arg-specific cysteine proteinases, which attack C5 and release biologically active C5a. C5a stimulates intracellular Ca^{2+} signaling which synergistically enhances the otherwise weak cAMP responses induced by TLR2 activation alone. Maximal cAMP induction requires the participation of CXC-chemokine receptor 4 (CXCR4), which is activated directly by the bacterium's fimbriae. The resulting activation of the cAMP-dependent protein kinase A (PKA) inactivates glycogen synthase kinase-3β (GSK3β) and inhibits the inducible nitric oxide synthase (iNOS)-dependent killing of the pathogen in macrophages. An additional pathway-induced downstream of TLR2 is an inside-out signaling pathway, mediated by RAC1, phosphatidylinositol-3 kinase (PI3K), and cytohesin 1 (CYT1), which transactivates complement receptor-3 (CR3). Activated CR3 binds *P. gingivalis* and induces extracellular signal-regulated kinase-1/ERK2 signaling, which in turn selectively downregulates IL-12 p35 and p40 mRNA expression through suppression of interferon regulatory factor 1 (IRF1). Inhibition of bioactive IL-12, and secondarily IFNγ, leads to impaired immune clearance of *P. gingivalis* [From Hajishengallis and Lambris (2011) Nat Rev Immunol 11:187–200 (used by permission)]

response, thereby leading to nonprotective and nonresolving chronic inflammation (Gaffen and Hajishengallis 2008; Kinane et al. 2006; Kumpf and Schumann 2008). Available evidence implicates the periodontal pathogen *Porphyromonas gingivalis* as a master of immune subversion (Hajishengallis 2009a) (Fig. 13.1). Indeed, *P. gingivalis* inhibits critical antimicrobial responses that could eliminate it, while on the other hand, stimulates local inflammation, which may facilitate nutrient acquisition (e.g., gingival crevicular fluid-derived hemin) and additionally cause collateral tissue damage (Hajishengallis et al. 2008; Coats et al. 2009; Wang et al. 2007; Burns et al. 2006; Hajishengallis et al. 2007; Slaney and Curtis 2008; Potempa and Pike 2009; Krauss et al. 2010). Recently, *P. gingivalis* was shown to act as a keystone pathogen which promotes the survival and virulence of the entire microbial community (Hajishengallis et al. 2011).

At least in principle, periodontitis could be inhibited by interventions aiming to control inflammation and counteract microbial subversion of the host response. This concept is discussed here in the context of the complement system, which is now recognized as a central network that orchestrates the

host response (Ricklin et al. 2010). Specifically, besides its classic antimicrobial functions (*see below*), complement crosstalks with and regulates other signaling systems, including Toll-like receptor (TLR) pathways (Hajishengallis and Lambris 2010). Despite its potentially host-protective role, however, complement forms a major link between infection and inflammatory pathology if overactivated or deregulated (Ricklin and Lambris 2007; Wagner and Frank 2010; Holers 2008; Ricklin et al. 2010).

13.2   Complement and Periodontal Disease

Besides the characteristic group of serum proteins (C1–9), the integrated complement system includes pattern-recognition molecules, convertases and other proteases, regulators, and receptors for interactions with immune mediators (Ricklin et al. 2010). The complement cascade can be triggered via distinct pathways (classical, lectin, or alternative) which converge at the third complement component (C3). The activation of the classical pathway is initiated by antigen-antibody complexes recognized by the C1q subunit of C1. The lectin pathway is triggered through interaction of the mannose-binding lectin, a secreted pattern-recognition molecule, with specific carbohydrate groups on microbial surfaces. Both the classical and lectin pathways proceed through C4 and C2 cleavage for the generation of the classical/lectin C3 convertase. The alternative pathway is initiated by low-level, spontaneous hydrolysis of C3 to $C3[H_2O]$, which forms the initial alternative pathway C3 convertase in the presence of factors B (fB) and D (fD). As long as there is no sufficient negative regulation (as in the case of bacteria or other nonself surfaces), this initiation is followed by rapid propagation of the alternative pathway involving an amplification loop (Dunkelberger and Song 2010; Ricklin et al. 2010). The alternative pathway can also be triggered by lipopolysaccharide and lipooligosaccharide in a way dependent upon the plasma protein properdin attached to bacterial surfaces (Spitzer et al. 2007; Kimura et al. 2008) and may potentially contribute to ≥80% of total complement activation (Harboe and Mollnes 2008). C3 activation by pathway-specific C3 convertases leads to the generation of effector molecules involved in (a) the recruitment and activation of inflammatory cells (e.g., the C3a and C5a anaphylatoxins that activate specific G-protein–coupled receptors, C3aR and C5aR [CD88], respectively), (b) microbial opsonization and phagocytosis (e.g., through the C3b opsonin), and (c) direct lysis of targeted pathogens (by means of the C5b-9 membrane attack complex [MAC]) (Ricklin et al. 2010). An alternative receptor for C5a is the C5a-like receptor 2 (C5L2; GPR77), which has been assigned both regulatory and proinflammatory roles (Ward 2009; Hajishengallis and Lambris 2010; Zhang et al. 2010; Bamberg et al. 2010).

At least in principle, local complement activation could promote periodontal inflammation through multiple pathways, including C5a-induced vasodilation, increased vascular permeability, and chemotactic recruitment of inflammatory cells, including neutrophils. In addition to their role in acute inflammation, neutrophils have been implicated in chronic inflammatory diseases (e.g., rheumatoid arthritis, inflammatory bowel disease, and chronic obstructive pulmonary disease) (Kasama et al. 2005; Kanazawa and Furukawa 2007; Simpson et al. 2009; Kitsis and Weissmann 1991). Neutrophils are also key effectors of inflammatory tissue injury in periodontitis (Serhan et al. 2008; Nussbaum and Shapira 2011) and can be found in great numbers in the gingival crevice (≥95% of total leukocytes) (Delima and Van Dyke 2003). Although gingival crevicular neutrophils form what looks like a "defense wall" against the periodontal bacteria, they largely fail to control the bacteria despite maintaining viability and capacity to elicit inflammatory responses (Delima and Van Dyke 2003; Lange and Schroeder 1971; Newman 1980; Schroeder and Listgarten 1997, Vitkov et al. 2010). The underlying reasons are largely unexplored.

Clinical and histological observations suggest that complement is indeed involved in periodontitis (Hajishengallis 2010). Chronically inflamed gingiva or gingival crevicular fluid from periodontitis patients displays increased levels of activated complement fragments relative to control samples from

healthy individuals (Patters et al. 1989; Nikolopoulou-Papaconstantinou et al. 1987; Rautemaa and Meri 1996; Beikler et al. 2008; Courts et al. 1977; Schenkein and Genco 1977; Niekrash and Patters 1986). Importantly, induction of experimental gingival inflammation in human volunteers causes progressive elevation of complement cleavage products correlating with increased clinical indices of inflammation (Patters et al. 1989).

Interestingly, a single nucleotide polymorphism of C5 (rs17611), which is associated with increased serum C5 levels and susceptibility to liver fibrosis and rheumatoid arthritis (Hillebrandt et al. 2005; Chang et al. 2008), was shown to be more prevalent in periodontitis patients than in healthy controls (Chai et al. 2010). Moreover, an immunohistochemical study showed weaker expression of CD59 in the gingiva of periodontitis patients compared to healthy controls, suggesting reduced protection of diseased tissues against autologous MAC-mediated tissue damage (Rautemaa and Meri 1996). A case of aggressive periodontitis accompanied by severe gingival angioedema was linked to dysregulated complement function, specifically C1INH deficiency (Roberts et al. 2003).

These studies suggest complement involvement in periodontal inflammation and pathogenesis. However, their correlative nature does not allow reliable conclusions as to the precise role(s) of the various complement pathways, nor do these observations necessarily imply that all complement pathways mediate destructive inflammation. In this regard, partial *C4* gene deficiencies are significantly more frequent in periodontal patients relative to healthy controls (Seppanen et al. 2007), therefore suggesting involvement of the classical and/or lectin pathway in a protective function. For instance, C3b generation via the C4-dependent classical and/or lectin pathways could promote opsonophagocytosis of periodontal bacteria, secondarily contributing to control of infection-induced inflammation. In conclusion, it has been uncertain which specific complement pathways need to be blocked to attenuate inflammatory pathology or kept intact to promote host defense. However, considerable insights have been gained by studies in preclinical models. At this point, there is sufficient evidence to implicate the C5a–C5aR axis in the pathogenesis of periodontitis (below).

13.3 Involvement of the C5a–C5aR Pathway in Periodontitis

The C5a anaphylatoxin is perhaps the most powerful effector molecule of the complement cascade, as it mediates chemotactic recruitment and activation of neutrophils and other inflammatory cells and is involved in synergistic complement interactions with Toll-like receptors (Guo and Ward 2005; Zhang et al. 2007). These immunostimulatory effects of C5a can potentially protect the host against microbial pathogens. In this regard, a major medical pathogen, *Staphylococcus aureus*, has evolved a strategy that allows it to block C5a binding and C5aR activation, via a secreted chemotaxis inhibitory protein (de Haas et al. 2004). Nevertheless, C5aR signaling can contribute to the pathogenesis of a number of acute or chronic inflammatory diseases, such as sepsis, acute lung injury, ischemia-reperfusion injury, and rheumatoid arthritis (Guo and Ward 2005; Okroj et al. 2007).

Intriguingly, in contrast to the *S. aureus* strategy, *P. gingivalis* is proactively involved in C5aR activation. Specifically, *P. gingivalis* employs its Arg-specific gingpains to generate biologically active C5a through limited degradation of C5, whereas the C5b remnant is proteolytically destroyed, ostensibly to prevent activation of the terminal complement pathway (Wingrove et al. 1992; Popadiak et al. 2007; Liang et al. 2011; Wang et al. 2010). *P. gingivalis* in fact can generate high levels of C5a (>30 nM) after a 30-min incubation in heat-inactivated human serum (Wang et al. 2010). This activity may appear to be counterproductive for the pathogen, given the important role of C5a in host defense. Strikingly, however, *P. gingivalis* was shown to exploit C5a to impair the killing function of macrophages via manipulation of specific signaling events in the absence of generalized immune suppression (Wang et al. 2010).

The mechanism involves synergistic production of high and sustained cAMP levels, which inhibit nitric oxide-dependent killing of *P. gingivalis* (Wang et al. 2010). This synergism requires a crosstalk between C5a-activated C5aR and *P. gingivalis*-activated TLR2, whereas downstream players include cAMP-dependent protein kinase A and glycogen synthase kinase-3β, the interplay of which inhibits the inducible nitric oxide synthase (Wang et al. 2010) (Fig. 13.1).

Moreover, the *P. gingivalis*-induced C5aR-TLR2 crosstalk regulates cytokine production that favors the pathogen (Liang et al. 2011). In this regard, the C5aR-TLR2 crosstalk inhibits TLR2-induced interleukin (IL)-12p70 which promotes immune clearance of *P. gingivalis* (Liang et al. 2011). In contrast, the same C5aR-TLR2 crosstalk upregulates inflammatory and bone-resorptive cytokines (IL-1β, IL-6, and TNF-α) which do not seem to harm *P. gingivalis*. These effects collectively lead to enhanced persistence of *P. gingivalis* in the host and set the stage for inflammatory tissue damage. This notion is supported by experimental periodontitis studies in the mouse model: Mice deficient in either C5aR or TLR2 were protected against *P. gingivalis*-induced inflammatory periodontal bone loss, whereas wild-type controls developed inflammation and suffer serious bone loss (Liang et al. 2011). In this inflammatory context, the proactive release of C5a by *P. gingivalis* could contribute to stimulation of inflammatory exudate for acquisition of nutrients like hemin and tissue breakdown products (peptides) which are essential for *P. gingivalis* and other asaccharolytic periodontal bacteria (Krauss et al. 2010). On the other hand, an isogenic mutant of *P. gingivalis*, which is deficient in all gingipain genes (KDP128), failed to persist in vivo and could not cause periodontitis (Liang et al. 2011; Hajishengallis et al. 2011). This difference in survival capacity may be related, at least in part, to the inability of KDP128 to generate C5a (Liang et al. 2011). These studies collectively indicate that the C5a–C5aR axis exerts a destructive role in periodontitis through a dual mechanism: (a) It is exploited by *P. gingivalis* to escape host defense, and (b) it mediates inflammatory periodontal bone loss.

## 13.4   C5aR-Targeted Intervention in Periodontitis

The above discussed findings provided a solid basis for rational C5aR-targeted intervention against *P. gingivalis* and periodontitis. This notion is supported by experimental evidence. Indeed, blockade of C5aR with a specific antagonist (Ac-F[OP(D)Cha-WR]; also known as PMX-53) abrogated the C5a-dependent subversive strategy of *P. gingivalis* and facilitated its immune clearance in vitro and in vivo (Wang et al. 2010; Liang et al. 2011). This effect may not necessarily imply protection against periodontal infection and inflammation in general, given the polymicrobial nature of periodontitis. However, it was recently shown that complement subversion by *P. gingivalis* can additionally benefit bystander periodontal bacteria in the same biofilm, which thereby displays quantitative and qualitative changes that lead to complement-dependent periodontitis at least in a mouse model (Hajishengallis et al. 2011) (Fig. 13.2).

Specifically, oral inoculation of mice with *P. gingivalis* exerts growth-enhancing effects and compositional changes in the oral microbiota, despite the very low colonization levels of *P. gingivalis* (<0.01% of the total bacterial counts) (Hajishengallis et al. 2011). Collectively, these actions lead to destructive inflammatory disease that requires the presence of the commensal microbiota and intact complement pathways since *P. gingivalis* fails to cause periodontitis in germ-free mice or conventionally raised mice deficient in C3aR or C5aR (Hajishengallis et al. 2011) (Fig. 13.3). Moreover, *P. gingivalis* fails to cause changes to the oral commensal microbiota of *C3aR*$^{-/-}$ or *C5aR*$^{-/-}$ mice, in contrast to normal specific pathogen-free (SPF) mice (Fig. 13.3).

The inability of *P. gingivalis* to alter the oral microbiota in *C5aR*$^{-/-}$ mice can be explained by the lack of C5aR, which is required by *P. gingivalis* to inhibit the killing capacity of leukocytes (Wang et al. 2010; Liang et al. 2011). *P. gingivalis*-affected leukocytes with impaired killing capacity would likely allow uncontrolled growth of other bacterial species in the same biofilm, accounting for the
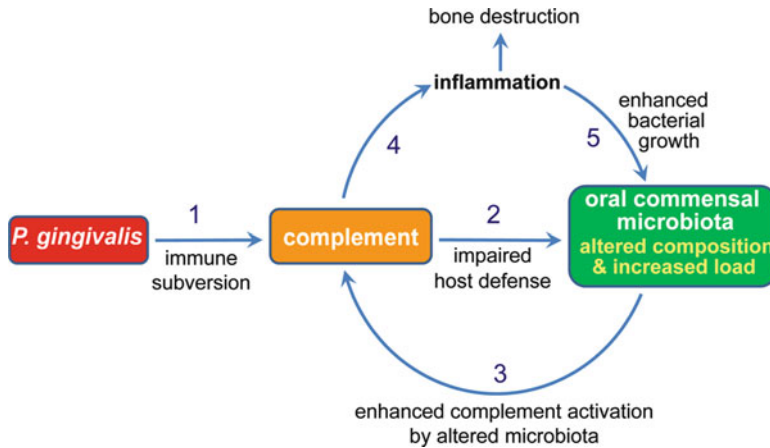
Fig. 13.2 *P. gingivalis* subverts complement leading to alterations in the oral microbiota and development of periodontitis. *P. gingivalis* impairs innate immunity through complement subversion leading to quantitative and qualitative changes to the oral commensal microbiota. Through complement, the altered microbiota causes inflammatory bone loss, whereas tissue breakdown products may further stimulate oral bacterial growth [From Hajishengallis et al. (2011) Cell Host Microbe 10:497–506 (used by permission)]
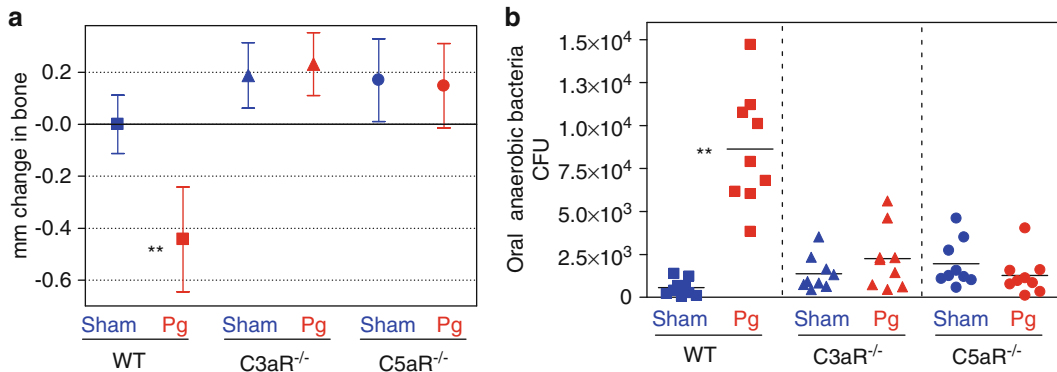
Fig. 13.3 *Complement-dependent elevation of the oral bacterial load and induction of bone loss.* BALB/c mice, either wild type (WT) or deficient in C3aR (*C3aR⁻/⁻*) or C5aR (*C5aR⁻/⁻*), were orally inoculated with *P. gingivalis* (Pg) or vehicle only (Sham) and assessed for bone loss (**a**) and levels of oral anaerobic bacteria (**b**). *Negative values* indicate bone loss relative to bone levels of the indicated controls (zero baseline), whereas *positive values* indicate increased bone levels. CFU counts are shown for each individual mouse with horizontal lines denoting mean values. **$p < 0.01$ versus corresponding control [From Hajishengallis et al. (2011) Cell Host Microbe 10:497–506 (used by permission)]

elevation of the total bacterial numbers. Consistent with the importance of gingipains in C5aR-dependent subversion of leukocytes (Liang et al. 2011), a gingipain-deficient isogenic mutant (KDP128) of *P. gingivalis* failed to elevate the oral bacterial load even in normal SPF mice. The C3aR requirement for the *P. gingivalis* effect on the oral microbiota and bone loss may be related to its synergistic interactions with C5aR that include reciprocal augmentation of receptor expression (Ricklin et al. 2010). In this regard, *P. gingivalis*-inoculated *C3aR⁻/⁻* mice displayed significantly reduced expression of C5aR (Hajishengallis et al. 2011).
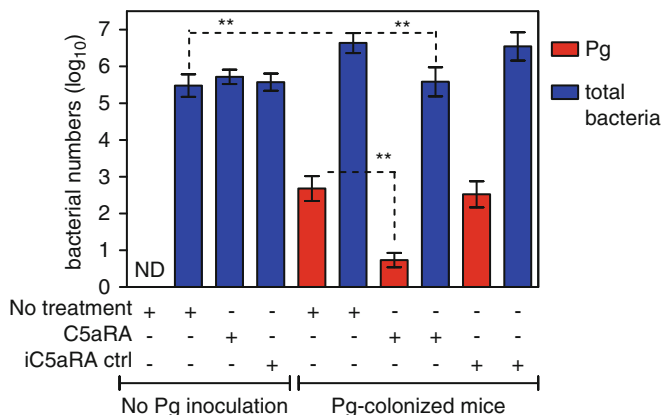
Fig. 13.4 Effects of C5aR antagonist (C5aRA), or inactive analog control (iC5aRA ctrl), on the numbers of *P. gingivalis* or total bacteria in the periodontal tissue of mice with or without previous inoculation with *P. gingivalis* Groups of mice were orally inoculated or not with *P. gingivalis* (as described above) and 7 days later, were injected with C5aRA or iC5aRA control, into the palatal gingiva, on the mesial of the first molar and in the papillae between first and second and third molars on both sides of the maxilla (1 μL of 1 μg per site; total of six sites). Two days later (day 9), the mice were sacrificed, and maxillary periodontal tissue was harvested to determine the levels of *P. gingivalis* colonization and the number of total oral bacteria, using quantitative real-time PCR of the *ISPg1* gene (*P. gingivalis*) or the 16 S rRNA gene (total oral bacteria). Two groups of mice, one of which was inoculated with *P. gingivalis*, were not treated with C5aRA or iC5aRA at day 7 and were sacrificed the same day for determining the levels of *P. gingivalis* and of total oral bacteria prior to the C5aRA or iC5aRA interventions. ND, Pg not detected. **p < 0.01 between indicated groups [From Hajishengallis et al. (2011) Cell Host Microbe 10:497–506 (used by permission)]

Since C5aR is required for the in vivo survival of *P. gingivalis* (Wang et al. 2010; Liang et al. 2011), local administration of a C5aR antagonist (PMX-53) could block the persistence of *P. gingivalis*, leading to its removal from the periodontal tissue and perhaps adversely affecting the total microbiota. Indeed, local administration of PMX-53 resulted in almost complete elimination of *P. gingivalis*, accompanied by a >10-fold reduction in the total numbers of oral anaerobic bacteria, which returned to their original lower levels (Hajishengallis et al. 2011) (Fig. 13.4). This reduction in the total microbiota was not a direct effect on the commensal bacteria by C5aRA since the antagonist failed to reduce the total oral bacterial numbers in mice not colonized with *P. gingivalis* (Fig. 13.4). These data clearly indicated that the experimental removal of *P. gingivalis* from the periodontal tissue, similar to its introduction, exerts a major influence on the oral microbiota. By analogy to the "keystone species" concept in macroecology, i.e., low-abundance species with a major supporting role for an entire ecological community (Power et al. 1996; Brown et al. 2001; Ebenman and Jonsson 2005), *P. gingivalis* may be regarded as such a species by fulfilling the criteria of low abundance and major influence on the microbial community. In fact, *P. gingivalis* could be characterized as a keystone pathogen, defined as "a keystone species which supports and shapes a microbial community in ways that also promote disease pathogenesis" (Hajishengallis et al. 2011).

13.5 Conclusions and Future Directions

Despite being a very minor constituent of the total oral microbiota, *P. gingivalis* can alter the numbers and community organization of the commensal bacteria, the presence of which is essential for inflammatory periodontal bone loss. Although PMX-53 has not been shown yet to inhibit periodontal bone loss after local administration, its capacity to block *P. gingivalis* persistence in the periodontal

tissue and to inhibit the overgrowth of the oral microbiota strongly suggests that it is a promising therapeutic against periodontitis. Moreover, C5aR inhibitors may have important therapeutic implications also in other infections or inflammatory diseases where *P. gingivalis* is thought to be implicated, such as oral aspiration pneumonia and atherosclerosis (Gibson et al. 2006; Okuda et al. 2005).

Despite this progress, additional systematic approaches are required to comprehensively identify the precise roles of the various complement pathways in the context of periodontal pathogenesis. Such information could reveal which specific pathways need to be blocked to reverse inflammatory pathology or, conversely, be enhanced (or left intact) to promote host defense. This would greatly facilitate complement-targeted therapeutic intervention against periodontitis.

Importantly, a number of complement-specific drugs are already in clinical trials for other inflammatory diseases (Ricklin and Lambris 2007; Wagner and Frank 2010). Among them, PMX-53, which blocks both mouse and human C5aR, has a good safety record when given orally to humans (Ricklin and Lambris 2007; Wagner and Frank 2010). This, and potentially other complement-targeted drugs, may prove effective as adjunctive treatments for periodontitis in the near future.

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