Pathogenic Microbes and Community Service Through Manipulation of Innate Immunity

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Abstract The periodontal pathogen *Porphyromonas gingivalis* undermines major components of innate immunity, such as complement, Toll-like receptors (TLR), and their crosstalk pathways. At least in principle, these subversive activities could promote the adaptive fitness of the entire periodontal biofilm community. In this regard, the virulence factors responsible for complement and TLR exploitation (gingipain enzymes, atypical lipopolysaccharide molecules, and fimbriae) are released as components of readily diffusible membrane vesicles, which can thus become available to other biofilm organisms. This review summarizes important immune subversive tactics of *P. gingivalis* which might enable it to exert a supportive impact on the oral microbial community.

Keywords Complement • C5a • TLR • Immune evasion • *P. gingivalis* • Biofilm • Periodontitis

1 Introduction

Innate immunity is a phylogenetically ancient system of host defense that represents the inherited resistance to infection (Janeway and Medzhitov 2002). It senses pathogens through "pattern recognition" or "missing-self recognition" strategies, e.g., through Toll-like receptors (TLRs) and complement, and triggers the activation of antimicrobial and inflammatory responses (Ricklin et al. 2010; Medzhitov 2007). TLRs recognize conserved microbial structures known as pathogen-associated molecular patterns (PAMPs) and, in general, different TLRs respond to distinct PAMPs (e.g., TLR2 recognizes lipoteichoic acid, TLR4 detects lipopolysaccharide, and TLR9 binds CpG DNA) (Beutler et al. 2006; Uematsu and Akira 2008). TLRs do not

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function in isolation but cooperate with other receptors in multireceptor complexes in lipid rafts of activated cells (Beutler et al. 2006; Hajishengallis et al. 2006; Triantafilou et al. 2002). Innate receptors shown to co-cluster with TLRs include CD14, CD36, CD55 (decay accelerating factor), complement receptor 3 (CR3; CD11b/CD18), CXC-chemokine receptor 4 (CXCR4), and growth differentiation factor 5 (GDF5) (Hajishengallis et al. 2006; Pfeiffer et al. 2001; Triantafilou et al. 2001). The formation of TLR-containing receptor clusters may serve to generate a combinatorial repertoire through which the host discriminates among the abundant and diverse microbial molecules and thereby to tailor the immune response. Moreover, TLR signaling pathways crosstalk with the complement system, which is now recognized to exert functions above and beyond simple pathogen tagging and elimination (Ricklin et al. 2010). For instance, effector molecules generated during the rapidly activated complement cascade (e.g., certain opsonins, anaphylatoxins, and sublytic concentrations of the terminal complement complex) stimulate signaling pathways that intercept and modify TLR-transduced signals (Hajishengallis and Lambris 2010). An emerging body of literature indicates that the TLR-complement interplay involves both synergistic and antagonistic interactions, which could, respectively, enhance host defense or regulate it to prevent excessive inflammation or autoimmunity (Hajishengallis and Lambris 2010; Hawlisch et al. 2005; Zhang et al. 2007).

Not surprisingly, given their importance in fighting infection, both complement and TLRs are key targets of immune evasion by successful pathogens, such as *Staphylococcus aureus*, *Helicobacter pylori*, vaccinia virus, and HIV (Lambris et al. 2008; Flannagan et al. 2009). Persistent infections and disease may also ensue when microbial pathogens successfully evade or subvert complement-TLR crosstalk pathways (Hajishengallis and Lambris 2010; Hajishengallis and Lambris 2011). By subverting innate immunity, pathogens may also undermine the overall host defense system, given the instructive role of innate immunity in the development of the adaptive immune response (Medzhitov 2001).

In this chapter, we focus on immune subversion mediated by the periodontal pathogen *Porphyromonas gingivalis*, placing particular emphasis on its newly identified crosstalk sabotage tactics. Immune evasion mechanisms of specific bacteria acquire their true relevance in the context of the collective virulence of the microbial communities in which they reside. In this regard, we examine published literature and discuss the potential of *P. gingivalis* to act as a k pathogen that might impact on the entire periodontal biofilm rendering it more pathogenic.

2 Keystone Pathogens in the Periodontal Biofilm

Porphyromonas gingivalis is a gram-negative anaerobic bacterium that is strongly associated with periodontitis, a chronic inflammatory disease that affects the tooth-supporting tissues (Pihlstrom et al. 2005). Periodontal disease affects the majority of the adult population, whereas an estimated 10–15% develops severe periodontitis. This is a condition that exerts a systemic impact on the patients who thereby

run increased risk for atherosclerotic heart disease, aspiration pneumonia, diabetes, adverse pregnancy outcomes, and perhaps rheumatoid arthritis (Xiong et al. 2006; Pihlstrom et al. 2005; Awano et al. 2008; Tonetti et al. 2007; de Pablo et al. 2009; Kebschull et al. 2010; Lundberg et al. 2010).

It should be noted that periodontitis is not initiated by a single bacterium but rather by certain species of subgingival gram-negative anaerobic bacteria, and the disease represents biofilm-induced destructive inflammation of the periodontal tissue (Davey and Costerton 2006; Gaffen and Hajishengallis 2008). Using a color-coded system, Socransky and colleagues characterized the periodontal microbial communities on the basis of cluster analysis, community ordination, and associated disease severity (Socransky et al. 1998). These communities are not random collections of bacteria, but organized and dynamic consortia that have evolved through mutually beneficial interactions, ultimately forming a niche with adequate nutrients and protection against host immunity. A high prevalence of red complex members, i.e., *P. gingivalis, Treponema denticola, and Tannerella forsythia,* correlates strongly with periodontal tissue destruction (Socransky et al. 1998; Holt and Ebersole 2005). *Prevotella intermedia, Fusobacterium nucleatum*, and *Aggregatibacter actinomycetmecomitans*, all members of the orange complex, are also associated with various forms of periodontal disease (Darveau 2009; Socransky et al. 1998). Although these and other putative periodontal pathogens have received particular attention, it should be noted that a sizeable portion of the approximately 700 bacterial species in the human oral cavity are as yet uncultivable (Kolenbrander et al. 2010). Despite its perceived complexity, the pathogenicity of the periodontal biofilm might be disproportionately dependent upon a select group of so-called "keystone" species. There is circumstantial evidence to suggest that at least one such species may be *P. gingivalis* (Darveau 2010; Hajishengallis 2009).

A keystone species is an organism that plays a crucial role in maintaining the structure of an ecological community, in a manner akin to the role of a keystone in an arch. Its impact on the community is greater than would be expected from its relative abundance. The "keystone species" concept was originally proposed by the ecologist R. T. Paine in 1969 (Paine 1969). A classic example of a keystone organism is the starfish *Pisaster ochraceus*. When this starfish was experimentally removed from a particular intertidal zone, this caused a dramatic reduction in biodiversity compared to a control area. In the context of microbial ecology, Darveau, defined the keystone species as one that "serves an essential function for the entire community, similar to a differentiated cell serving a function for an entire tissue" (Darveau 2010). The implication here is not that the periodontal biofilm would necessarily collapse in the absence of a single important species. Rather, the pathogenicity of a biofilm missing a keystone member could be attenuated to the point of not being capable of driving destructive periodontal inflammation. A keystone microbe is not necessarily a pathogen. The human gut symbiont *Bacteroides thetaiotaomicron* imparts stability to the gut ecosystem and might be considered as a keystone species (Backhed et al. 2005). One of its mechanisms, in this context, involves manipulation of host gene expression. If, for example, dietary polysac-

charides become scarce, *B. thetaiotaomicron* induces gut epithelial cell production of fucosylated glycans on which bacteria can feed (Backhed et al. 2005). The modification of either dietary conditions or of immune selective pressure in ways that stabilize the microbiota could be thus considered as keystone functions.

3 *Porphyromonas gingivalis:* Interfering with Host Immunity for the Microbial "Common Good"?

Porphyromonas gingivalis is employing an array of virulence factors, such as cysteine proteinases (known as gingipains), lipopolysaccharide (LPS), and fimbriae (Table 1), through which it manipulates innate immunity in an effort to promote its adaptive fitness.

In addition to its established role in periodontitis, *P. gingivalis* is also a common isolate from aspiration pneumonia and lung abscesses (Okuda et al. 2005; Finegold 1991) and has been detected in a viable state in atherosclerotic plaques (Kozarov et al. 2005). Its role in cardiovascular disease has been the focus of a number of mechanistic and epidemiological studies (Kebschull et al. 2010). There is also intriguing, though still circumstantial, evidence that the ability of *P. gingivalis* to citrullinate bacterial and host proteins may provide a molecular mechanism for generating antigens that drive the autoimmune response in rheumatoid arthritis (Lundberg et al. 2010). Of course the potential dental and medical importance of *P. gingivalis* does not necessarily render it a keystone species. A keystone species should express virulence factors that could provide "community service". Below we discuss virulence factors of *P. gingivalis* that manipulate innate immunity in ways that could benefit additional periodontal species.

3.1 The LPS as a Tool to Antagonize TLR4

Although *P. gingivalis* is a gram-negative bacterium that expresses LPS, its lipid A moiety presents some unusual features that make it invisible to TLR4 or, even worse for the host, antagonistic to TLR4 (Fig. 1). Specifically, the organism coordinately utilizes specific lipid A 1- and 4′-phosphatases and a deacylase to generate a tetra-acylated and dephosphorylated lipid A structure (Coats et al. 2009). This modification renders the LPS biologically inert, thereby allowing *P. gingivalis* evade TLR4 activation (Coats et al. 2009). The presence of high concentrations of hemin (becomes abundant under inflammatory conditions) suppresses lipid A 1-phosphatase activity and leads to the generation of a mono-phosphorylated lipid A, which actively antagonizes TLR4 activation (Coats et al. 2009; Coats et al. 2005). Although TLR4 could be activated in periodontal tissue by LPS from other bacteria (e.g., by *A. actinomycetemcomitans*, the LPS of which is potent TLR4 ago-

Table 1 Immune subversion mechanisms of *P. gingivalis* and virulence factors involved

Mechanism	Virulence factors	References
Inhibition of complement activation through degradation of the central complement component (C3)	Gingipains (especially HRgpA and RgpB)	(Popadiak et al. 2007; Potempa et al. 2009)
Hijacking complement regulatory proteins (C4b-binding protein)	Gingipain (HRgpA)	(Potempa et al. 2008)
Inhibition of phagocyte killing via C5aR–TLR2 crosstalk	Gingipains (HRgpA, RgpB)	(Wang et al. 2010)
Suppression of TLR2-induced IL-12 by *P. gingivalis*-generated C5a	Gingipains (HRgpA, RgpB)	(Liang et al. 2010)
Degradation of TLR coreceptors (CD14), cytokines (IL-12, IL-1β, IL-6, IFN-γ), or antimicrobial peptides (e.g., LL-37)	Gingipains (HRgpA, RgpB, Kgp)	(Potempa and Pike 2009)
Inherent resistance to complement-mediated lysis	LPS with anionic polysaccharide repeat units	(Slaney et al. 2006; Rangarajan et al. 2008)
TLR4 evasion	Dephosphorylated tetraacylated lipid A LPS	(Coats et al. 2009)
TLR4 antagonism (e.g., inhibition of TLR4-induced β-defensin expression)	Monophosphorylated tetraacylated lipid A LPS	(Coats et al. 2009; Coats et al. 2005; Darveau 2010)
Upregulation of negative regulators of TLR signaling (IRAK-M) in monocytes	LPS	(Domon et al. 2008)
Inhibition of phagocyte killing via CXCR4–TLR2 crosstalk	Fimbriae	(Hajishengallis et al. 2008)
Suppression of TLR2-induced IL-12 via CR3 outside-in signaling	Fimbriae	(Hajishengallis et al. 2007)
Promotion of intracellular survival via CR3-mediated entry into macrophages	Fimbriae	(Wang et al. 2007)

nist), this activity is likely to be attenuated by *P. gingivalis* LPS. Proof-of-concept evidence was obtained in *in vitro* studies (Hajishengallis et al. 2002; Darveau et al. 2002). The underlying mechanism involves competitive TLR4 blocking mediated by the binding of the antagonistic *P. gingivalis* lipid A to MD2, a TLR4-bound protein required for TLR4 responsiveness to LPS (Coats et al. 2005). This mechanism may not only help other bacteria in close vicinity to *P. gingivalis* but also those in remoted areas of mixed species biofilms. Indeed, *P. gingivalis* releases LPS-bearing outer membrane vesicles (OMVs) that can readily diffuse in the gingival crevice (free space between the teeth and the gingiva) or even penetrate gingival tissue (Darveau 2010; Lamont and Jenkinson 1998). The *P. gingivalis* OMVs may therefore undermine TLR4-dependent innate immunity for the microbial community at

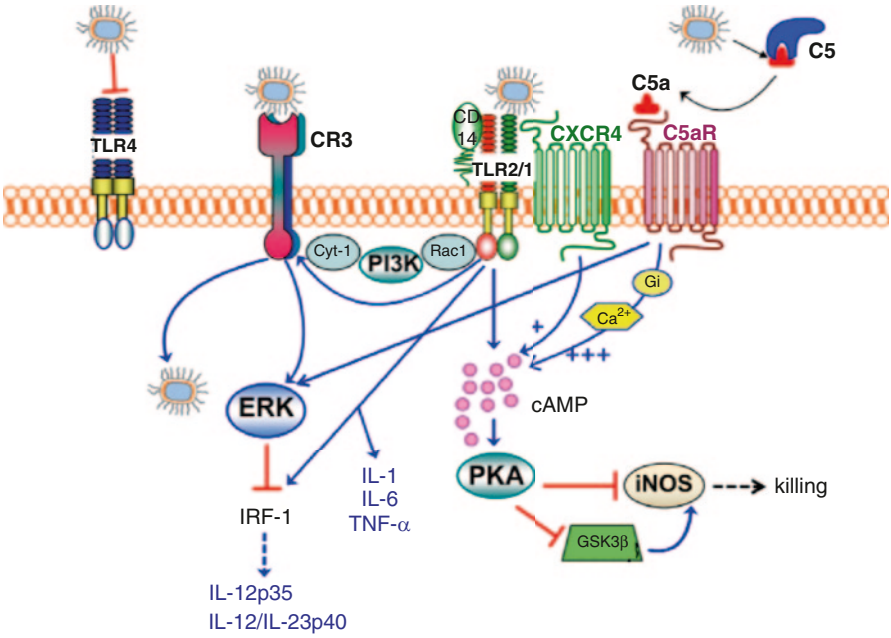

Fig. 1 Subversive crosstalk pathways exploited by *P. gingivalis*. In macrophages, *P. gingivalis* interacts with the CD14/TLR2/TLR1 receptor complex (Hajishengallis et al. 2006). Moreover, *P. gingivalis* uses its gingipains to attack C5 and release biologically C5a (Wingrove et al. 1992; Wang et al. 2010). Upon C5aR binding, C5a stimulates Gαi-dependent intracellular Ca^{2+} signaling which synergistically enhances the otherwise weak cAMP responses induced by TLR2/TLR1 activation alone. Maximal cAMP induction is achieved by the participation of another G protein-coupled receptor, the CXCR4, which interacts directly with *P. gingivalis* and coassociates with both TLR2 and C5aR in lipid rafts (Hajishengallis et al. 2008; Wang et al. 2010). The ensuing activation of the cAMP-dependent protein kinase A (PKA) pathway inactivates glycogen synthase kinase-3β (GSK3β) and impairs the inducible nitrogen synthase (iNOS)-dependent killing of the pathogen in macrophages *in vitro* and *in vivo* (Wang et al. 2010). The interaction of *P. gingivalis* with TLR2/TLR1 also activates inside-out signaling, propagated by Rac1, PI3 K, and cytohesin-1 (Cyt-1), which induces the high-affinity conformation of CR3 (Hajishengallis and Harokopakis 2007; Harokopakis and Hajishengallis 2005). CR3 then binds and internalizes *P. gingivalis*; this is a relatively safe portal of entry since CR3 is not linked to vigorous microbicidal mechanisms (Rosenberger and Finlay 2003; Wang and Hajishengallis 2008). The CR3-*P. gingivalis* interaction also leads to induction of ERK1/2 signaling. This in turn downregulates IL-12 p35 and p40 mRNA expression (Hajishengallis et al. 2007), possibly through suppression of a critical transcription factor (the interferon regulatory factor 1 [IRF1]) that is required for IL-12 expression (Hawlisch et al. 2005). This inhibitory ERK1/2 pathway is also activated downstream of the C5aR. The suppressive effects of CR3 and C5aR on TLR2-induced cytokine production are selective for IL-12 and do not affect induction of other proinflammatory cytokines (e.g., IL-1β, IL-6, and TNF-α) *in vitro* or *in vivo*. Inhibition of bioactive IL-12 by these mechanisms results in impaired immune clearance of *P. gingivalis in vivo* (Hajishengallis et al. 2007; Liang et al. 2010). *P. gingivalis* interacts with at least one more TLR, the TLR4, which, however, is proactively antagonized by an atypical lipopolysaccharide lipid A moiety (Coats et al. 2005)

large, such as inhibition of β defensin expression by epithelial cells (Lu et al. 2009). The *P. gingivalis* OMVs are not solely decorated with LPS but contain additional virulence factors including gingipains and fimbriae, both of which are implicated in immune evasion (Amano et al. 2010; Hajishengallis 2009) (Table 1). These factors are discussed below.

3.2 The Gingipains as a Means to Manipulate Complement and Leukocytes

P. gingivalis can suppress all three mechanisms of complement activation, i.e., the classical, lectin, and alternative pathways, through proteolytic degradation of key complement components such as the C3 (Potempa and Pike 2009). These subversive activities are mediated by the gingipains and particularly the Arg-specific enzymes.

When gingipain-deficient mutants are exposed to human serum, active complement fragments are readily deposited on the bacterial surface; strikingly, however, the mutants maintain full viability as does the wild-type organism (Slaney et al. 2006). This observation was conclusively attributed to the expression of a surface anionic polysaccharide (APS) which confers exquisite resistance to complement-mediated lysis (Slaney et al. 2006). This molecule was later identified as a novel type of LPS, termed A-LPS (with APS repeating units) to differentiated it from the better known O-LPS (with O-antigen tetrasaccharide repeating units) (Rangarajan et al. 2008). However, an intriguing question arising from this discovery is the following: What is the point of inhibiting complement when the pathogen is already intrinsically resistant to complement-mediated lysis? A possible explanation is that *P. gingivalis* has evolved to inhibit complement activation, not for its own protection, but for the benefit of companion species sharing the same subgingival niche.

The capacity of *P. gingivalis* to inhibit complement becomes all the more puzzling considering that it has developed ways to directly and "voluntarily" activate specific complement components. For instance, the Arg-specific gingipains of *P. gingivalis* function as C5 convertase-like enzymes (Wingrove et al. 1992) and the bacterium generates high levels of C5a *in vitro* and *in vivo* (Wang et al. 2010; Liang et al. 2010). This is quite curious for a pathogen which goes at great lengths to inhibit all three known initiation mechanisms of complement activation (Popadiak et al. 2007; Krauss et al. 2010). However, there is something unique about C5a and *P. gingivalis*. The pathogen exploits this anaphylatoxin (but not C3a) to instigate a subversive crosstalk between the C5a receptor (C5aR; CD88) and TLR2, and in doing so it escapes killing (Liang et al. 2010; Wang et al. 2010). It appears that since *P. gingivalis* does not antagonize TLR2 at the receptor level as it does with TLR4 (see above), it has instead opted to instigate crosstalk signaling between TLR2 and other innate receptors to undermine innate immunity (Hajishengallis et al. 2008; Wang et al. 2007; Hajishengallis et al. 2007; Wang et al. 2010) (Fig. 1). The crosstalk between C5aR and TLR2 may be facilitated by the ability of *P. gingivalis* to in-

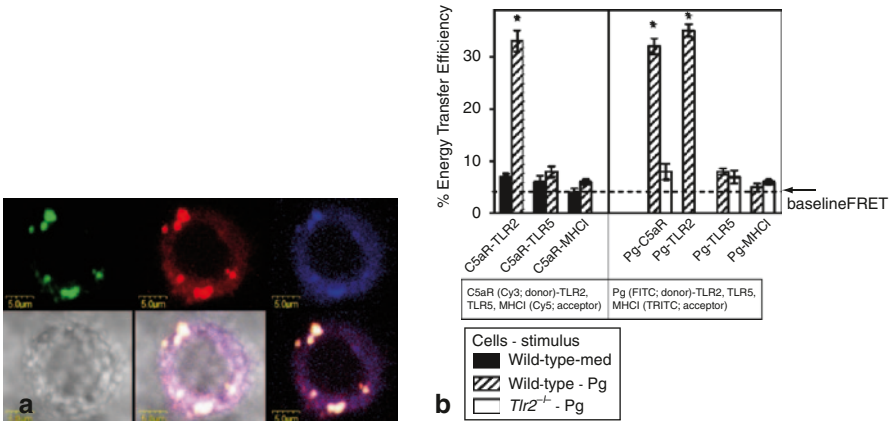

Fig. 2 ***P. gingivalis*** **induces colocalization of C5aR and TLR2 in macrophages. a** Confocal colocalization of *P. gingivalis* (*green*), C5aR (*red*), and TLR2 (*blue*). Bottom right, merged image. **b** Fluorescence resonance energy transfer between the indicated donors and acceptors measured from the increase in donor (Cy3 or FITC) fluorescence after acceptor (Cy5 or TRITC) photobleaching. Data are means ± SD ($n = 3$). *, $p < 0.01$ between the indicated groups and controls. FRET analysis has revealed significant energy transfer between Cy3-labeled C5aR and Cy5-labeled TLR2 in *P. gingivalis*-stimulated but not resting macrophages, whereas no significant energy transfer was detected between Cy3-labeled C5aR and Cy5-labeled TLR5 or MHC Class I (controls). Moreover, significant energy transfer was observed between FITC-labeled *P. gingivalis* and TRITC-labeled C5aR or TLR2 (but not TLR5 or MHC Class I). C5aR appeared to associate with *P. gingivalis* in a TLR2-dependent way since the *P. gingivalis*-C5aR FRET association was abrogated in *Tlr2*$^{-/-}$macrophages. (The data are from the reference Wang et al. 2010) (Used by permission)

duce a coassociation between these receptors. Indeed, confocal microscopy has revealed colocalization of the two receptors in *P. gingivalis*-stimulated macrophages (Fig. 2a), while fluorescence resonance energy transfer experiments have shown that C5aR, TLR2, and *P. gingivalis* come into molecular proximity(Fig. 2b) (Wang et al. 2010).

Mechanistically, upon binding the C5aR, C5a stimulates Gαi-dependent intracellular Ca^{2+} signaling which synergistically enhances an otherwise weak cAMP response induced by *P. gingivalis*-induced TLR2 activation alone. In this crosstalk pathway, sustained elevated production of cAMP leads to the activation of the cAMP-dependent protein kinase A which inactivates the glycogen synthase kinase-3β and impairs nitric oxide-dependent killing of *P. gingivalis* in macrophages (Wang et al. 2010). Crosstalk between C5aR and TLR2 also inhibits the ability of neutrophils to kill *P. gingivalis*, although the underlying signaling pathway is cAMP-independent (Hajishengallis et al. 2010). *In vivo*, the *P. gingivalis*-induced C5aR–TLR2 crosstalk regulates cytokine production in favor of the pathogen (Liang et al. 2010). Specifically, this oral bacterium proactively and selectively inhibits TLR2-induced interleukin (IL)-12p70 which is required for its immune clearance, whereas the same C5aR–TLR2 crosstalk upregulates other inflammatory and bone-resorptive cytokines (IL-1β, IL-6, and TNF-α) which do not seem to harm *P. gingivalis*. These effects would be expected to enhance the persistence of *P. gingivalis* in the host

and to create favorable conditions for inflammatory tissue damage. Indeed, this notion is consistent with observations that mice deficient in either C5aR or TLR2 are protected against *P. gingivalis*-induced inflammatory periodontal bone loss (Liang et al. 2010). Moreover, an isogenic mutant of *P. gingivalis* which is deficient in all gingipain genes (KDP128) fails to persist *in vivo*, in contrast to the wild-type organism which promotes its survival in a C5aR-dependent way (Liang et al. 2010). This difference in survival capacity may be related, at least in part, to the inability of KDP128 to generate C5a (Liang et al. 2010).

These subversive effects are unlikely to benefit only *P. gingivalis*. If neutrophils or macrophages are rendered impotent in terms of killing, the same leukocytes would also be unable to control bystander bacteria cohabiting the same niche with *P. gingivalis*. It is often assumed that biofilms are inherently resistant to phagocytosis. However, this is an unproven assumption. In fact, neutrophils can potentially recognize and respond to biofilms through phagocytosis, degranulation (e.g., release of lactoferrin and elastase) and formation of extracellular DNA traps (Meyle et al. 2010). Therefore, when biofilms successfully resist elimination, this is likely to involve proactive microbial mechanisms that evade or subvert phagocytosis or other leukocyte functions. Time-lapse video microscopy and confocal laser scanning microscopy has revealed that, depending on the nature and composition of the biofilm, neutrophils can move into biofilms and eat up bacteria as they move across, or display a relatively immobile phenotype with limited phagocytosis in their immediate vicinity (Guenther et al. 2009; Gunther et al. 2009; Meyle et al. 2010; Wagner et al. 2007). Moreover, neutrophils can detect quorum-sensing molecules of bacteria which will attract them to the developing biofilm and stimulate their killing mechanisms (Wagner et al. 2007). In the human gingival crevice, recruited neutrophils form what looks like a “defense wall” against the dental biofilm bacteria; intriguingly, however, the neutrophils largely fail to control the bacteria despite maintaining viability and capacity to elicit inflammatory responses, including release of extracellular DNA traps (Delima and Van Dyke 2003; Lange and Schroeder 1971; Newman 1980; Schroeder and Listgarten 1997; Vitkov et al. 2010; Ryder 2010). The mechanistic basis for the relative impotence of crevicular neutrophils to control periodontal infection and to become involved in non-resolving inflammation is largely unexplored, but could be due to a number of mechanisms. At least in part, this may be the result of the *P. gingivalis* capacity to generate high levels of C5a and exploit it for targeted suppression of the leukocyte killing function without affecting—in fact, enhancing—the overall inflammatory response (Hajishengallis 2010).

In addition to their role in complement manipulation, the gingipains (but not host proteases) were shown to modify the cell surface of healthy neutrophils masquerading them as apoptotic, thereby leading to their removal from active duty by fellow macrophages. The underlying mechanism involves a dual action; firstly, proteolytic cleavage of an antiphagocytic signal (CD31) and, secondly, the generation of a novel, but not yet adequately characterized, “eat-me” signal on the neutrophil surface (Guzik et al. 2007). The fact that gingipains are present in the readily diffusible OMVs released by *P. gingivalis* may allow additional periodontal bacteria to benefit from gingipain-mediated immune subversion.

3.3 *Fimbriae: Multitasking Beyond Colonization*

Fimbriae are adhesive hair-like appendages emanating from the bacterial cell surface (Lamont and Jenkinson 1998; Hajishengallis 2007). The major fimbriae of *P. gingivalis* are encoded by the *fimA* operon and are indispensable for colonization and host cell invasion (Lamont and Jenkinson 1998; Hajishengallis 2007). Moreover, recent evidence has implicated fimbriae in immune evasion. Specifically, *P. gingivalis* uses its fimbriae to bind CR3 which induces extracellular signal-regulated kinase 1/2 signaling that in turn selectively inhibits mRNA expression of the p35 and p40 subunits of IL-12 (Hajishengallis et al. 2007) (Fig. 1). IL-12 is a key cytokine involved in pathogen clearance through regulatory effects on the production of interferon (IFN)-γ, which is a potent activator of the macrophage microbicidal capacity (Trinchieri 2003). Consistent with the above *in vitro* findings, wild-type mice elicit lower levels of IL-12 and IFN-γ and display impaired clearance of *P. gingivalis* infection compared to mice that lack CR3 (Hajishengallis et al. 2007). Similar results are seen after CR3 blockade with a specific antagonist (Hajishengallis et al. 2007). Importantly, the interaction of *P. gingivalis* or its purified fimbriae with CR3 inhibits the ability of LPS from other bacteria, such as *A. actinomycetemcomitans*, to induce IL-12p70 and IFN-γ in mouse macrophages or human monocytes (Hajishengallis et al. 2007).

The fimbriae of *P. gingivalis* can additionally co-ligate the CXC-chemokine receptor 4 (CXCR4) and TLR2 in lipid rafts leading to crosstalk signaling that inhibits the killing capacity of macrophages (Hajishengallis et al. 2008). Macrophages can be found in relatively low numbers in the gingival crevice but they are consistently present in the underying junctional epithelium (Tonetti et al. 1994). In addition, macrophages can interact with invading periodontal bacteria in the connective tissue of the periodontium. When *P. gingivalis* fimbriae are released as components of OMVs, they may allow additional periodontal species evade killing through CR3 and CXCR4 exploitation. These notions, if experimentally confirmed, would substantiate the concept of community service by a single, keystone species.

4 Is the Presence of *P. gingivalis* Benefiting Other Periodontal Bacteria?

A widely used model of experimental periodontitis involves the implantation of *P. gingivalis* in the oral cavity of mice by oral gavage (Graves et al. 2008). Although sometimes regarded as a monoinfection model, this may not actually be so unless germ-free mice are used (Graves et al. 2008). The indigenous oral microbiota of mice contains bacteria with the potential to become pathogenic for the periodontium under conditions of disrupted periodontal homeostasis. For example, mice with impaired mobilization of leukocytes to sites of infection, owing to combined P- and E-selectin deficiency, display massive oral bacterial colonization and induction of

gingival inflammation and alveolar bone loss (Niederman et al. 2001). Similar bacteriological findings and periodontitis features are observed in mice deficient in lysosomal-associated membrane protein-2; in these mice, the neutrophils are readily mobilized to the periodontal tissues but display impaired bacterialkilling and are thus unable to control their indigenous oral flora (Beertsen et al. 2008). In both cases, induction of inflammation and periodontal bone loss is prevented by antibiotics, thus further confirming the involvement of indigenous bacteria in the disease (Beertsen et al. 2008; Niederman et al. 2001).

In a rabbit model of *P. gingivalis*-induced periodontitis, the implantation of *P. gingivalis* "stimulated" a shift to a more anaerobic flora in the dental biofilm and an overall increase in bacterial load (Hasturk et al. 2007). For example, significantly higher levels were seen for *Prevotella intermedia*, *Eikenella corrodens*, and *Peptostreptococcus micros*, whereas previously undetected species like *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum* were readily observed (Hasturk et al. 2007). When resolvin E1 was topically applied to inhibit the inflammatory process, this treatment also resulted in the elimination of *P. gingivalis*, possibly attributed to the inability of this asaccharolytic organism to secure nutrients under non-inflammatory conditions. In this regard, the inflammatory exudate that bathes the gingival crevice is a rich source of peptides (e.g., from degradation of host proteins or tissue breakdown products) and iron (derived from hemin) (Delima and Van Dyke 2003; Krauss et al. 2010). Interestingly, following the elimination of *P. gingivalis*, the resident oral flora returned to its baseline composition and numbers, i.e., those present before the implantation of *P. gingivalis* (Hasturk et al. 2007). We have also noticed that the introduction of *P. gingivalis* in the mouse oral cavity is associated with a dramatic increase in the total number of anaerobic oral bacteria (unpublished observations). These findings have two implications. Firstly, the observed bone loss may not be attributed solely to *P. gingivalis*. Secondly, *P. gingivalis* appears to act as a keystone species, in that its presence imparts a positive influence on the dental biofilm. However, if this action is mediated via manipulation of innate immunity, one would expect that the effect would be abrogated in animals lacking the hijacked innate receptor(s).

Although the mechanisms underlying the "keystone species" concept are poorly understood, initial observations in simple models may provide some mechanistic insight. As indicated above, *P. gingivalis* can protect itself from leukocyte killing by capitalizing on a subversive crosstalk between C5aR and TLR2 (Liang et al. 2010; Wang et al. 2010; Hajishengallis and Lambris 2011). This is not a mechanism that can be employed by just any oral bacterium and, in fact, *F. nucleatum* is susceptible to killing by leukocytes. However, *F. nucleatum* displays a dramatic resistance to leukocyte killing in the presence of *P. gingivalis* (Fig. 3). Simple co-infection models, such as the chamber model of host-microbe interactions (Graves et al. 2008), may prove helpful in identifying mechanisms of collective resistance to host defenses mediated by putative keystone species. This seemingly altruistic behavior of keystone organisms may not be quite so, considering that the benefited bacteria may reciprocate the favor. In this regard, the colonization of *P. gingivalis* is facilitated by other members of the mixed-species periodontal biofilm and *F. nucleatum* is likely

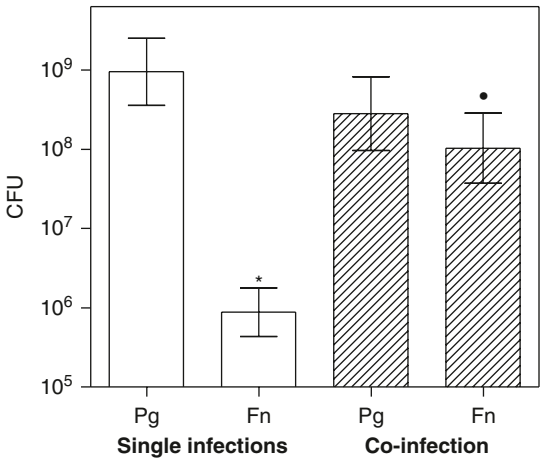

Fig. 3 The presence of *P. gingivalis* enhances the survival of other periodontal species. *P. gingivalis* ATCC 33277 and *F. nucleatum* ATCC 25586 (Fn) were injected into subcutaneously implanted chambers in mice, either alone (10^9 CFU) or together (5×10^8 CFU each), and 24 h postinfection chamber fluid was aspirated to determine viable CFU counts. The data are means ±SD ($n=5$ mice). *, $p<0.01$ between Fn and Pg CFU when inoculated alone; •, $p<0.01$ between CFU counts of the same organism recovered from single infections and co-infection

one of them (Kolenbrander et al. 2010). Interestingly, oral co-infection of mice with *F. nucleatum* and *P. gingivalis* induces increased bone loss compared to either organism alone (Polak et al. 2009). The underlying mechanisms may involve mutually beneficial interactions in addition to immune subversion, such as interbacterial adherence and metabolic cooperation.

5 Perspectives and Conclusions

In the course of evolution, successful pathogens have "learned" to breach innate defense systems such as complement and TLRs (Roy and Mocarski 2007; Lambris et al. 2008) but also, as exemplified here with *P. gingivalis*, to exploit their crosstalk pathways (Hajishengallis et al. 2008; Wang et al. 2010; Hajishengallis and Lambris 2011). These subversive strategies (Fig. 1) as well as other important evasion tactics (Hajishengallis 2009; Potempa and Pike 2009; Yilmaz 2008) (Table 1) may explain, at least in part, the ability of *P. gingivalis* to persist and establish chronic infections. There is also growing circumstantial evidence suggesting the potential of *P. gingivalis* to act as a keystone pathogen, i.e., one that could promote the survival and persistence of other members of the periodontal biofilm community (Darveau 2010; Hajishengallis 2009; Hasturk et al. 2007) (Fig. 3). Briefly stated, bacteria lacking protective or evasive mechanisms on their own may still thrive in the presence of *P. gingivalis*. A synergy of shared, communal mechanisms of immune evasion, colo-

nization, and nutrient procurement should maximize the survival potential and virulence of the whole community. In this context, various combinations of periodontal bacteria, such as *P. gingivalis*, *T. forsythia*, *T. denticola*, *F. nucleatu,* and *A. actinomycetemcomitans* become more virulent in *in vivo* models of infection than the individual organisms alone (Jenkinson and Lamont 2005; Feuille et al. 1996; Kesavalu et al. 1998; Kesavalu et al. 2007; Polak et al. 2009; Kinane and Hajishengallis 2009). *P. gingivalis*-centred ecosystems may perturb otherwise homeostatic host-bacterial interactions, thereby leading to non-protective and non-resolving chronic inflammation in the periodontium. Future research to elucidate the mechanisms by which *P. gingivalis* subverts immunity and promotes the collective virulence of the periodontal biofilm would facilitate the rational design of therapeutic interventions against human periodontitis.

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