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Porphyromonas gingivalis-Host Interactions: Open War or Intelligent Guerilla Tactics?

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Abstract

This review summarizes and discusses virulence mechanisms whereby *Porphyromonas gingivalis* can persist in the oral cavity. It is proposed that that the virulence of *P. gingivalis* is dependent, at least in part, upon its ability to establish a complex host-pathogen molecular crosstalk which subverts innate immunity. The sophisticated stealth and sabotage tactics used by *P. gingivalis* may additionally benefit co-habiting organisms occupying the same niche

Keywords

P. gingivalis; host-pathogen interactions; innate immunity; inflammation; immune evasion

1. Introduction

Porphyromonas gingivalis is a gram-negative anaerobic and asaccharolytic rod that is widely recognized as a predominant contributor to human periodontitis [1]. This is a polymicrobial infection-driven inflammatory disease of the oral cavity, characterized by chronicity and destruction of the tooth-supporting tissues. Moreover, *P. gingivalis* is implicated as an accessory factor in certain systemic conditions, such as atherosclerotic heart disease or aspiration pneumonia [2]. This pathogen is perhaps the most intensively studied oral organism at the molecular level and its pathogenicity is attributed to a panel of potential virulence factors, such as cysteine proteinases (gingipains), hemagglutinins, lipopolysaccharide (LPS), and fimbriae, *i.e.*, adhesive hair-like appendages emanating from the bacterial cell surface [3]. These and other virulence molecules are thought to coordinately enable *P. gingivalis* to colonize or invade host tissues and secure critical nutrients [3].

P. gingivalis isamong the late colonizers of the oral cavity. Its colonization is influenced by saliva, which serves as a vector for its transmission and initial entry into the oral environment. Moreover, the salivary pellicle-coated tooth surfaces provide anchoring points for *P. gingivalis* fimbriae, which additionally display binding affinity for certain early colonizing bacteria [3]. These interactions presumably allow the pathogen to attach to a solid substratum, thereby preventing its clearance by the salivary flow. Later in the infection process, *P. gingivalis* may reach its eventual niche, *i.e.*, the subgingival crevice (space between the free gingiva and the tooth surface), by proliferation and spreading or by relocation of dislodged bacteria. In addition to providing attachment sites, early colonizers, such as facultative

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anaerobic streptococci, may facilitate *P. gingivalis* colonization by reducing oxygen tension to levels permissive for obligate anaerobic growth [3].

However, the capacity of a pathogen to secure an appropriate niche and persist requires more than simply possessing virulence factors for tissue adherence and nutrient procurement. To establish a chronic infection in a hostile host environment, it is imperative that pathogens find ways to evade or subvert host defense mechanisms aiming to eliminate them. Microbes which successfully undermine the host response appear to preferentially target innate immunity [4], partly because these are the defense mechanisms first encountered by the pathogens. In addition, it should be appreciated that subversion of the innate response may also disable the overall host response, given the instructive role of innate immunity in the development of adaptive immunity [5]. In this review, I summarize and discuss molecular virulence mechanisms of *P. gingivalis* (Table 1) and conclude that its pathogenicity is, at least in part, dependent upon sophisticated stealth and sabotage tactics.

2. Staying alive in the crevice: interactions with epithelial cells and neutrophils

In addition to tooth surfaces, gingival sulcular epithelial cells offer additional attachment sites for *P. gingivalis* in the crevicular region. However, *P. gingivalis* goes beyond simple adhesion and actively invades and replicates within epithelial cells in vitro [3]. This strategy may allow limited exposure to the extracellular space and could thus shield the pathogen from humoral immune surveillance. Importantly, human crevicular epithelial cells do harbor intracellular *P. gingivalis*, as well as other periodontal pathogens [6]. To extend its intracellular stay, *P. gingivalis* inhibits gingival epithelial cell apoptosis, at least in part, by secreting an ATP-hydrolyzing enzyme that suppresses ATP-induced apoptosis [7]. Although there may be conditions under which *P. gingivalis*-infected gingival epithelial cells may succumb to apoptosis (reviewed in ref. [8]), the pathogen may then implement "plan B" involving safe exit and intercellular spreading [7]. This mechanism may potentially prolong its intracellular lifestyle.

Clinical periodontal observations have suggested that induction of inflammatory bone resorption requires a minimum distance (< 2.5 mm) between the bone and the bacteria, and this proximity may occur upon bacterial invasion of the gingival connective tissue (reviewed in ref. [9]). To keep the bacteria at bay, neutrophils may chemotactically migrate into the crevicular region for combined intracellular and extracellular killing. However, the ability of gingival epithelial cells to induce interleukin (IL)-8 for chemoattraction and activation of neutrophils is proactively inhibited by *P. gingivalis* in a cell-invasion dependent manner [10]. This so-called "local chemokine paralysis" is but one mechanism whereby *P. gingivalis* attempts to suppress or delay neutrophil influx into its crevicular niche. Indeed, *P. gingivalis* LPS does not induce endothelial cell expression of E-selectin and, furthermore, inhibits E-selectin upregulation by other periodontal bacteria [11]. Inhibition of E-selectin expression results in diminished neutrophil adhesion to endothelial cells in vitro [11] and, presumably, could suppress diapedesis and migration to sites of infection. In vivo, however, *P. gingivalis* LPS does induce E-selectin expression, albeit at significantly lower levels compared to enterobacterial LPS [12]. Apparently, the interactions of *P. gingivalis* with several cell types in vivo may result in a different net effect than seen in vitro with isolated endothelial cells and, eventually, neutrophils do migrate into the gingival crevicular region. However, the pathogen possesses protective mechanisms to overcome the oxidative stress environment generated by the neutrophil release of toxic reactive oxygen species [13]. In fact, *P. gingivalis* is exquisitely resistant to killing by the oxidative burst [14]. The neutrophils may thus resort to non-oxidative means in an effort to control *P. gingivalis*, although the pathogen seems to suppress at least some of these mechanisms (*e.g.*, degradation of the cathelicidin LL-37) [15]. The in vitro

opsonization of *P. gingivalis* with high-affinity specific antibody facilitates its killing by neutrophils, although antibody production in periodontitis appears to be of low affinity and of questionable protective value [16]. In conclusion, the neutrophil killing of *P. gingivalis* is, by no means, an easy task, and not without collateral damage, since release of reactive oxygen species can contribute to periodontal tissue destruction.

3. Fooling cell-mediated immunity

Although the aforementioned intracellular sequestration tactics may protect *P. gingivalis* from humoral immunity (*e.g.*, antibodies), they may not confer protection against cell-mediated immunity, which involves participation of natural killer cells, antigen-specific cytotoxic T-cells, and macrophages. Indeed, at least in principle, *P. gingivalis*-infected epithelial cells could be killed by cytotoxic T cells or natural killer cells, the activation of which is positively regulated by macrophage-derived IL-12 [17]. Intriguingly, *P. gingivalis* actively suppresses the production of bioactive IL-12 (IL-12p70) in macrophages [18]. Specifically, *P. gingivalis* interacts via its fimbriae with complement receptor-3 (CR3) leading to activation of extracellular signal-regulated kinase 1/2, which in turn selectively inhibits IL-12 production by at least 60% in vitro and in vivo [18] (Fig. 1). Even if this is a leaky mechanism, successfully produced IL-12 may be functionally inactivated through proteolytic degradation, as is also the case with a selected few more cytokines [15]. It is conceivable that the capacity of *P. gingivalis* to inhibit or destroy IL-12 may suppress efficient activation of cytotoxic T cells and natural killer cells, thereby favoring *P. gingivalis* survival in permissive cells, such as epithelial cells. This notion has yet to be specifically addressed experimentally, *e.g.*, by using *P. gingivalis*-infected cells as targets in cytotoxicity assays.

On the other hand, there is evidence that CR3-dependent inhibition of IL-12 promotes *P. gingivalis* survival [18], perhaps through regulatory effects on the production of IFN-γ, which is a potent activator of the macrophage microbicidal capacity [17]. Indeed, wild-type mice elicit lower IL-12 and IFN-γ levels and display impaired clearance of *P. gingivalis* systemic infection compared to CR3-deficient mice [18]. Similar results were observed upon CR3 blockade with a specific antagonist which, moreover, inhibited the ability of *P. gingivalis* to persist in the mouse oral cavity and to induce periodontal bone loss [18]. Thus, if left uninhibited, *P. gingivalis* may cause disease via suppression of IFN-γ-dependent cell-mediated immunity. It is of interest to note here that, unlike classical enterobacterial LPS, the LPS of *P. gingivalis* is a poor inducer of IFN-γ even when it is administered in vivo [19]. *P. gingivalis* therefore appears to prevent induction of IFN-γ through both active and passive means.

The potential of *P. gingivalis* to interfere with cell-mediated immunity is further supported by its direct immunosuppressive effects on gene expression in $CD4^+$ and $CD8^+$ T cells in mice [16]. Several studies in humans have also suggested that periodontal bacteria actively suppress T cell-mediated immunity and, thereby, presumably contribute to periodontal lesion development [16]. However, there is not, as yet, direct evidence for this notion from human studies, which tend to be more correlative than mechanistic, due to several limitations including important ethical considerations.

4. Modulation of the Toll-like receptor (TLR) response

In general, most virulence factors employed by pathogens for undermining the host response are proteins. Unlike the relatively invariant microbe-associated molecular patterns (*e.g.*, lipoteichoic acid, LPS, peptidoglycan), most of which perform essential microbial physiologic functions, virulence proteins are responsible for microbial adaptation within a particular host environment and are thus characterized by mutability [5]. However, the molecular armamentarium used by *P. gingivalis* to manipulate the host response includes its LPS, *i.e.*, a structure that could be thought of as relatively invariable. In this regard, *P. gingivalis* expresses

a heterogeneous mixture of atypical lipid A structures, including species that trigger TLR2 signaling, weakly stimulate TLR4, or potently antagonize TLR4 activation [20]. Strikingly, the *P. gingivalis* lipid A structure is regulated in response to microenvironmental hemin concentrations which, in turn, are influenced by inflammation [21]. At a mechanistic level, it is thought that high levels of hemin result in upregulation of deacylase activity which converts penta-acylated lipid A into tetra-acylated lipid A [21]. Interestingly, *P. gingivalis* penta-acylated lipid A activates TLR4, whereas tetra-acylated lipid A antagonizes TLR4 [20]. It could be speculated, therefore, that the regulated heterogeneity of its LPS structures may enable *P. gingivalis* to manipulate the innate response and suppress TLR4-mediated immunity [20]. This intriguing possibility awaits experimental confirmation, although it should be noted that evasion of TLR4 activation has been associated with increased virulence of another organism. Specifically, genetic modification of *Yersinia pestis* to produce a hexa-acylated lipid A structure (and thus a strong TLR4 agonist) results in a completely attenuated strain, as opposed to the highly virulent wild-type organism which synthesizes tetra-acylated lipid A with poor TLR4-agonistic activity [22].

Another possible mechanism to manipulate TLR signaling, at least in macrophages, involves the ability of *P. gingivalis* LPS to markedly upregulate the IL-1R-associated kinase (IRAK)-M, a negative regulator of the TLR signaling pathway [23]. This mechanism may contribute to inhibition of the innate response, a notion that is certainly consistent with observations that *P. gingivalis* LPS is a relatively poor inducer of IL-1β and TNF-α production compared with *E. coli* LPS [3]. The *P. gingivalis* gingipains may also contribute, albeit indirectly, to reduced TLR activation through their ability to degrade CD14, an important coreceptor of TLR2 and TLR4 [15].

Unlike TLR4, TLR2 (in association with its signaling partner TLR1) can readily recognize and respond to *P. gingivalis*. However, the pathogen can manipulate TLR2 signaling by instigating a molecular cross-talk between this TLR and the CXC-chemokine receptor 4 (CXCR4) [24] (Fig. 1). Specifically, the binding of *P. gingivalis* fimbriae to CXCR4 induces cAMP-dependent protein kinase A signaling, which in turn suppresses TLR2/1-mediated NF-κB activation and induction of nitric oxide production. The potential importance of this mechanism is supported by in vivo observations. Indeed, mice treated with a specific CXCR4 antagonist display increased production of nitric oxide and enhanced ability to control a systemic infection with *P. gingivalis* compared to control mice [24]. This and the above cited examples suggest that the manipulation of the TLR response is a likely mechanism whereby *P. gingivalis* may promote its adaptive fitness in the mammalian host.

5. Scoffing at complement or making good use of it?

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 [15]. As a further precaution, the pathogen hijacks a physiological inhibitor of complement activation. Specifically, the C4b-binding protein is captured on the bacterial cell surface and thereby interferes with the activation of the complement cascade [15]. 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 [25]. This observation was conclusively attributed to expression of a surface anionic polysaccharide which confers exquisite resistance to complement-mediated lysis [25]. However, as is often the case, new discoveries only breed new questions: What is the survival advantage of being capable to inhibit complement when the pathogen is intrinsically resistant to complement-mediated killing? A possible explanation is that this apparent redundancy seen in in-vitro experiments may actually

represent an essential combination of strategies for effective protection in vivo. Another possibility could be that the surface anionic polysaccharide is present only in selected strains of *P. gingivalis*. Although this structure was initially identified in W50 (a Type IV *fimA* strain) and 381 (a Type I *fimA* strain) [25], subsequent work has shown that a specific monoclonal antibody is reactive with a variety of distinct strains, suggesting that the anionic polysaccharide is a general feature of *P. gingivalis* (M.A. Curtis, personal communication). Given that this surface glycan provides inherent protection, an alternative interpretation is that *P. gingivalis* has evolved the ability to inhibit complement activation, not for its own protection, but for the benefit of companion species sharing the same subgingival niche. This seemingly altruistic behavior may be justified in terms of evolutionary survival advantage for *P. gingivalis*, since the benefiting bacteria appear to reciprocate. In this regard, the pathogen depends on other members of the mixed-species periodontal biofilm for enhanced colonization and full expression of virulence [26].

Intriguingly, the overall strategy of *P. gingivalis* may not aim to simply inactivate the complement cascade, but also to actively exploit specific aspects of it for interfering with innate immunity in general. In this regard, *P. gingivalis* proactively generates an active complement fragment through direct action of its Arg-specific gingipains. Specifically, the pathogen causes limited degradation of C5 resulting in functional C5a, *i.e.*, it is chemotactically active as is the physiologically produced C5a anaphylatoxin [27]. Moreover, *P. gingivalis* Arg-specific gingipains may indirectly generate functional C5a through their ability to activate prothrombin to form thrombin [28]. Thrombin, in turn, generates biologically active C5a by acting as a C5 convertase [29]. It is quite safe to assume that a successful pathogen would not likely engage in activities that may promote host defense, unless the net outcome offers some survival advantage. It, therefore, becomes important to understand and interpret this superficially suicidal behavior of *P. gingivalis* which appears to generate weapons (C5a) for the host. C5a interacts with two receptors, the classical C5a receptor (C5aR; CD88) and the recently identified and modestly characterized C5L2, although it is the interaction with C5aR which mediates the anaphylactic and chemotactic activities of C5a [30]. Interestingly, C5aR-induced signaling in macrophages interferes with TLR4-mediated production of IL-12, and this leads to suppression of Th1 cell-mediated immunity in vitro and in vivo [31,32]. It could, therefore, be speculated that *P. gingivalis* causes the generation of active C5a fragment for inhibiting Th1 cell-mediated immunity. Nevertheless, *P. gingivalis* has already at least one mechanism for promoting its survival through inhibition of IL-12 and IFN-γ in vivo, which is dependent upon CR3 hijacking [18] (see also above). However, if C5a is exploited by *P. gingivalis* for the same reason, this may not be a redundant but rather a complementary mechanism as the interaction of CR3 with *P. gingivalis* does not fully inhibit IL-12 production [18]. The possibility for C5a exploitation is also supported by observations that the intracellular survival of *P. gingivalis* in phagocytes is promoted in the presence of C5a, although the underlying mechanism is yet unclear (our unpublished observations). Future studies will determine whether *P. gingivalis* displays a purely defensive agenda in facing the complement system, or whether it proactively exploits specific complement components for undermining other innate immune mechanisms.

6. Macrophage lipid rafts: Safe portals for *P. gingivalis*?

Certain intracellular pathogens, such as *Salmonella typhimurium*, *Shigella flexneri*, and *Mycobacterium* spp. enter macrophages through lipid rafts, *i.e.*, cholesterol- and sphingolipid-enriched membrane microdomains which partition receptors for various cellular signaling and trafficking processes [33]. The “choice” of lipid rafts as portals of pathogen entry might appear ironic, given their role in host defense as signaling platforms for TLRs and other immune receptors [34]. Nevertheless, lipid rafts appear to be targeted by pathogens. It is thought that the lipid-raft route of microbial uptake affords protection from the intracellular degradative lysosomal pathway (reviewed in ref. [33]). This is attributed to the notion that internalized rafts

do not readily fuse with late endosomes and lysosomes [35], in sharp contrast to opsonic phagocytosis through Fc receptors, which is raft-independent and readily leads to pathogen degradation [33]. Recent evidence suggests that *P. gingivalis* may also exploit lipid rafts for immune evasion. Specifically, the pathogen interacts directly with lipid rafts on the cell surface of macrophages and uses them to enter the cells in a way that increases its survival potential [36]. Disruption of lipid rafts by cholesterol depletion partially suppresses the uptake of *P. gingivalis*, which however now traffics preferentially to the lysosomes and is killed [36]. These data suggest that *P. gingivalis*-containing phagosomes originating from lipid rafts have a more favorable intracellular fate than phagosomes emanating from non-raft regions of the cell membrane.

However, what remains uncertain is whether *P. gingivalis* entry via lipid rafts is sufficient per se to confer protection from lysosomal trafficking and killing. Alternatively, the passing of *P. gingivalis* from lipid rafts may offer an opportunity to exploit or subvert raft-mediated signaling. In this context, mycobacteria appear to exploit lipid rafts in a very specific way. Indeed, to prevent post-phagocytosis killing, mycobacteria induce recruitment of a coat protein designated TACO (tryptophane aspartate-containing coat protein; also known as coronin-1) which associates in a cholesterol-dependent way with the phagosomalmembrane and prevents its fusion with lysosomes [33]. Arguably, CR3 may be at least one of potential receptors exploited by *P. gingivalis* in lipid rafts. First, CR3 is recruitable to lipid rafts upon activation with appropriate microbial stimuli, including *P. gingivalis* fimbriae [34]. Second, CR3-independent phagocytosis of *P. gingivalis* (due to CR3 blockade or genetic deficiency) dramatically inhibits its intracellular survival compared to conditions that allow CR3-mediated entry [37]. Mechanistically, this could be accounted for by observations that CR3 is not linked to vigorous microbicidal mechanisms [4]. This, however, begs the question of why the host expresses an Achilles' heel type of phagocytic receptor. A plausible explanation is that, under physiologic conditions, CR3 is heavily committed with phagocytosis of apoptotic cells (reviewed in ref. [18]). Since apoptotic cells are not normally recognized as danger, induction of a vigorous host response is not justified, and *P. gingivalis* aptly exploits this CR3-associated loophole (Fig. 1).

Although the lipid raft route of entry promotes the intracellular survival of *P. gingivalis*, the pathogen does not seem capable of replicating within macrophages [36,37] as it does in epithelial or endothelial cells [3,38]. This raises questions as to the biological significance of this entry mechanism. An intriguing possibility may be suggested in the context of the periodontitis-atherosclerosis connection [2] and the observation of viable *P. gingivalis* in atherosclerotic plaques [39]. Conceivably, the ability of *P. gingivalis* to persist within macrophages may allow this organism to exploit them as "Trojan horses" for relocation to systemic tissues and subsequent infection of permissive cells (*e.g.*, endothelial cells). Although this is a hypothetical scenario at the moment, the capacity of *P. gingivalis* for cell exit and infection of new host cells is well documented [7].

7. Stealth attack or overt aggression?

As discussed above, *P. gingivalis* attempts to downregulate or even deregulate the host response using a variety of documented or suspected strategies. However, the pathogen does induce inflammatory reactions and, after all, it is strongly associated with a chronic disease (periodontitis) where tissue damage is mostly mediated by inflammatory mechanisms. This represents an apparent paradox begging for explanation (Table 2). It could be speculated, however, that mechanisms for immunosuppression or promotion of inflammation may be employed under different conditions, depending on whether inflammation is beneficial or not for *P. gingivalis*. For example, in the early stages of infection, *P. gingivalis* may suppress host defense to facilitate its colonization of the subgingival crevice. At later stages, when a relatively

recalcitrant pathogenic biofilm has been established, stimulation of inflammatory serum exudate may contribute to an increased demand for nutrients, such as hemin, a source of essential iron. Although this is an interesting hypothesis, it is currently unknown whether and how this strategy could be coordinated. Nevertheless, the observation that *P. gingivalis* alters the lipid A structure of its LPS [21], suggests that, at least in principle, the proinflammatory potential of this pathogen is a regulatable activity.

Another possibility is that *P. gingivalis* may not induce a wholesale immunosuppression but rather exploits selective signaling pathways in ways that undermine the ability of the host to eliminate it; in this scenario, pathways eliciting nonproductive inflammation stay intact and cumulatively contribute to periodontal tissue destruction. For example, the interaction of *P. gingivalis* with CR3 inhibits induction of IL-12, which would contribute to its clearance [18], but promotes TNF-α [18], which contributes to periodontal bone loss [9]. Still another possibility, which might appear heretical, is that periodontal tissue destruction may predominantly be the result of inflammation caused by endogenous inducers of inflammation, such as breakdown products of the extracellular matrix [40], which can readily be generated by *P. gingivalis* [15].

The putative evasion tactics of *P. gingivalis* contrast it with aggressive pathogens which engage in “open war” with the host, but also with most commensals, which homeostatically stimulate immune responses under an “armed peace” deal with the host. The relationship of *P. gingivalis* with the host could be equated to a “guerilla war” using stealth and/or sabotage tactics. In that sense, *Aggregatibacter actinomycetemcomitans*, another important periodontal pathogen, appears to be a more overt pathogen than *P. gingivalis*. While *P. gingivalis* inhibits induction of IL-12 and IFN-γ-dependent cell-mediated immunity [18], *A. actinomycetemcomitans* is a powerful inducer of IL-12 and IFN-γ [41]. This activity is predominantly dependent on the LPS of *A. actinomycetemcomitans* which does not appear to modify the TLR reactivity of its lipid A as, by contrast, occurs in *P. gingivalis* (discussed above). Moreover, contrary to the *P. gingivalis*-instigated “local chemokine paralysis” [10], *A. actinomycetemcomitans* actually induces production of IL-8 and expression of intercellular adhesion molecule-1 in gingival epithelial cells [42]. Therefore, far from inhibiting, *A. actinomycetemcomitans* appears to stimulate the recruitment of neutrophils. Whereas *P. gingivalis* may survive by suppressing the initiation of the host response, *A. actinomycetemcomitans* may promote its persistence by elaborating powerful toxins (*e.g.*, leukotoxin and cytolethal distending toxin) capable of killing innate and adaptive immune effector cells (reviewed in ref. [43]). These contrasting behaviors do not rule out synergistic enhancement of virulence through combined immunosuppression of selective pathways and direct killing of effector cells, which is likely to happen in polymicrobial infections like periodontitis.

Despite evidence suggesting an immunosuppressive role for *P. gingivalis*, is it possible that this species, like the ancient Roman god Janus, displays two faces? One that is sneaky and stealthy and another of a more aggressive character, each represented by distinct strains? In this regard, it should be noted that most studies focusing on molecular virulence mechanisms of *P. gingivalis* have predominantly used Type I fimbriated strains (*e.g.*, 33277 and the related 381). These studies collectively suggest that Type I fimbriae play critical roles in colonization, cell invasion, and subversion of the host response [3,24]. However, the *fimA* gene, which encodes for the major fimbrillin subunit, exhibits considerable sequence diversity and *P. gingivalis* fimbriae have been classified into six genotypes (I–V and Ib) [44]. Recent functional comparative studies have shown that Type II fimbriated *P. gingivalis* (Pg-II) is dramatically more proinflammatory than Pg-I, both in vitro and in vivo [44,45]. Moreover, in systemic models of disseminated infection, Pg-II (as well as Pg-IV) are considerably more aggressive and cause more tissue damage than Pg-I [46,47]. This contrasts with findings that Pg-I is more

virulent than Pg-II in the mouse periodontitis model, where colonization of the oral cavity is a formidable challenge and may be compromised if the implanted strain elicits a vigorous host response (our unpublished observations). The above discussed FimA-based virulence differences still hold when the Type I and II *fimA* genes were exchanged between Pg-I and Pg-II strains, resulting in two swap mutants expressing the heterologous FimA [45](and our unpublished data), suggesting that the type of fimbriae dictate the behavior of *P. gingivalis* to a significant degree.

It is conceivable that different *P. gingivalis* strains may have developed distinct virulence strategies to cope with the mammalian host. For example, certain strains, such as Pg-I, may not afford to be overtly aggressive due to lack of protective capsule (in this regard, most Pg-II strains are encapsulated while Pg-I strains like 33277 and 381 are not [48]). Overt pathogens may often succeed by overwhelming innate defenses, whereas organisms lacking such capacity may have instead evolved stealth strategies to evade elimination by the immune system. A recent study has identified a great number of *P. gingivalis* mobile genetic elements which appear to be involved in extensive genome rearrangement and generation of many strain-specific protein-coding sequences [49]. It is intriguing to speculate that the virulence diversity of different *P. gingivalis* strains may work in synergy to promote the survival of the species. In this regard, a single periodontal site can harbor multiple *P. gingivalis* sequence types and different *fimA* genotypes appear to colonize the same site [50]. It is conceivable, although it remains to be established, that the combination of immunosuppressive activities and frontal attack strategies by different strains may be more effective for *P. gingivalis* survival than when either strategy acts alone.

8. Concluding remarks

The published information reviewed here suggests that, at least in principle, *P. gingivalis* has evolved a sophisticated program of tactics to evade multiple checking points of the innate immune system. Through its gingipains, fimbriae, LPS, or other factors, *P. gingivalis* appears capable to manipulate innate recognition mechanisms (Fig. 1), find refuge in relatively safe environments, evade or subvert complement, and in general proactively modify the innate response in ways that favor its persistence in the host. Some of the putative mechanisms may simply be potentialities, although at least some have been confirmed in vivo in animal models (Table 1). However, it is now appreciated that *P. gingivalis* is genetically diverse and includes strains that appear to thrive on alternative, more direct offensive tactics (Table 2; point 4). Whether “immunosuppressive” and “aggressive” strains fight for survival independently or combine their diverse tactics has yet to be addressed experimentally. Of course, periodontitis is not a mono-infection but rather a polymicrobial disease caused by a pathogenic biofilm residing in the subgingival region. Although *P. gingivalis* is but one of many periodontal pathogens, its presence may endow the whole biofilm with a panoply of critical virulence attributes; these may additionally promote the survival of other bacteria in the mixed species biofilm of the periodontal pocket. Understanding how *P. gingivalis* evades, subverts, or attacks the immune system is of paramount importance to understanding its role in periodontitis and associated systemic diseases and developing effective intervention therapeutic strategies.

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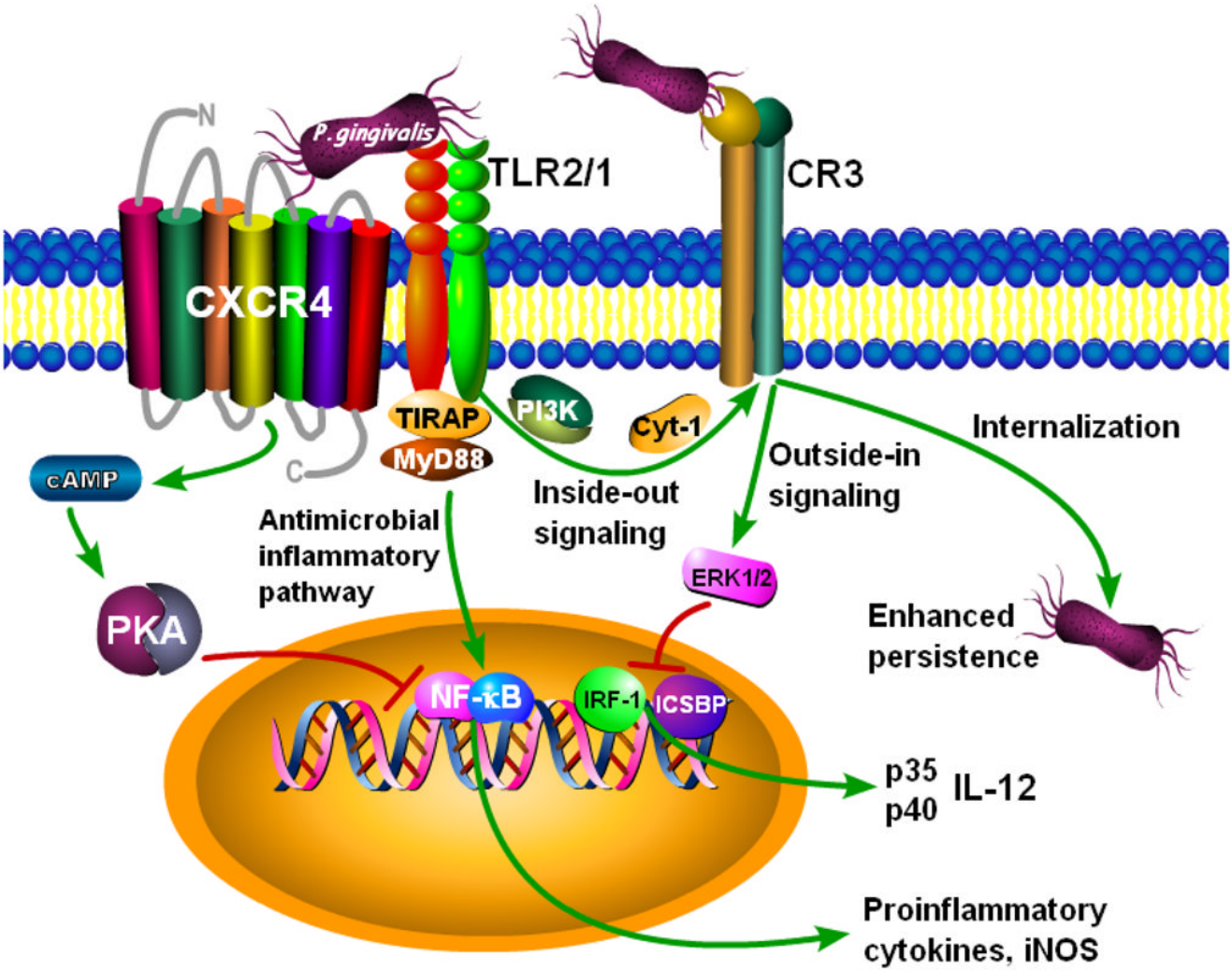

Fig. 1. Model of *P. gingivalis* exploitation of innate immune receptors for undermining host defenses

P. gingivalis is predominantly recognized by the TLR2/1 heterodimer [34]. Through its fimbriae, the pathogen binds CXCR4 which cross-talks with and suppresses the TLR2/1-induced TIRAP/MyD88-mediated antimicrobial pathway [24]. The mechanism involves CXCR4-induced activation of cAMP-dependent protein kinase A (PKA) which suppresses NF-κB activation and induction of proinflammatory cytokines and antimicrobial molecules, such as the inducible nitric oxide synthase (iNOS). These inhibitory effects promote *P. gingivalis* survival in vitro and in vivo [24]. The CXCR4 mechanism does not however inhibit the *P. gingivalis*-induced inside-out signaling, which proceeds via phosphatidylinositol 3-kinase (PI3K) and cytohesin-1 and activates the high-affinity conformation of CR3 [18,37] (and unpublished data). Intriguingly, *P. gingivalis* interacts with activated CR3 and is thereby internalized; this is a relatively safe portal of entry since CR3 is not linked to vigorous microbicidal mechanisms [4,36]. Moreover, the *P. gingivalis*-CR3 interaction induces outside-in signaling, which via extracellular signal-related kinase 1/2 (ERK1/2) downregulates IL-12 p35 and p40 mRNA expression [18], possibly through ERK1/2 suppression of IRF-1 and ICSBP transcription factors [31]. At the protein level, the outcome is reduced production of bioactive IL-12 resulting in impaired immune clearance of *P. gingivalis* in vivo [18].

Table 1

Molecular virulence mechanisms of *P. gingivalis*

Mechanisms	Key virulence factor	Outcome	Type of evidence	Refs.
Structural modification of the lipid A structure of LPS; conversion of penta-acylated lipid A (TLR4 agonist) to tetra-acylated lipid A (TLR4 antagonist)	Deacylase	Suppression of TLR4 activation	in vitro	[20,21]
Upregulation of negative regulators of TLR signaling (IRAK-M) in monocytes	LPS	May contribute to inhibition of the innate response	in vitro	[23]
Induction of TLR2 inside-out signaling for CR3 activation in macrophages	Fimbriae	**a.** CR3-dependent inhibition of IL-12 **b.** CR3-mediated entry leading to enhanced survival	in vitro& in vivo	[18,37]
Instigation of CXCR4/TLR2 cross-talk in macrophages	Fimbriae	Suppression of immune clearance of bacteria	in vitro & in vivo	[24]
Degradation of essential TLR coreceptors (CD14), cytokines (IL-12, IL-1β, IL-6, IFN-γ), or antimicrobial peptides (*e.g.*, LL-37)	Gingipains	May suppress innate immunity	in vitro	[15]
Intrinsic resistance to the lytic action of complement	Cell-surface anionic polysaccharide	Resistance to complement-mediated killing	in vitro	[25]
Inhibition of complement activation (classical, lectin, and alternative pathways)	Gingipains (particularly Arg-specific)	Inhibition of deposition of active complement fragments on the bacterial cell surface	in vitro	[15]
Lipid raft-mediated entry into macrophages	Not determined [1]	Reduced intracellular killing	In vitro	[37]
Counteraction of oxidative damage	Rubrerythrin (nonheme iron protein), alkyl hydroperoxide reductase, FeoB2 (ferrous iron transport protein)	Resistance to environmental oxidative stress and oxidative killing by phagocytes	In vitro and in vivo [2]	[13,14]
ATP hydrolysis	Nucleoside diphosphate kinase	Suppression of ATP-induced epithelial cell apoptosis; enhanced intracellular persistence	in vitro	[7]
Inhibition of IL-8 production by epithelial cells	Requires cell invasion	Possible suppression or delay of neutrophil influx	in vitro	[10]
Lack of E-selectin induction; inhibition of E-selectin	LPS	Diminished neutrophil adhesion to endothelial cells; presumably suppressed	in vitro; not confirmed in vivo [3]	[11,12]

Mechanisms	Key virulence factor	Outcome	Type of evidence	Refs.
upregulation by other periodontal bacteria		migration to sites of infection.		
Immunosuppressive effects on gene expression in $CD4^+$ and $CD8^+$ T cells	Outer-membrane antigens	May suppress cell-mediated immunity [4]	In vivo	[16]

[1] Only wild-type bacteria used; surface structures such as fimbriae or LPS which interact with lipid raft-associated receptors may be important.

[2] In vivo evidence obtained for ruberythrin; in vivo testing did not support a role for alkyl hydroperoxide reductasel; FeoB2 not tested in vivo.

[3] Although *P. gingivalis* LPS did induce E-selectin expression in vivo, this was a relatively weak activity compared to enterobacterial LPS.

[4] Cell-mediated immunity may also be inhibited through inhibition of IL-12 and IFN-γ, as indicated above.

Table 2

Reconciliation of *P. gingivalis* (Pg) immunosuppressive phenotype with chronic inflammation.

	Proposed mechanisms	Theoretical basis* [ref.]
1	Regulation of proinflammatory potential according to circumstances. **a.** Early-stage immunosuppression to facilitate colonization. **b.** Later-stage inflammation to meet increased demand for nutrients (*e.g.*, hemin in inflammatory exudate).	Pg regulates its proinflammatory potential, at least in part by altering its lipid A structure [21].
2	Selective immunosuppression. **a.** Killing mechanisms are inhibited **b.** Nonproductive inflammation that fails to eliminate Pg remains intact and contributes to tissue destruction.	Pg-activated CR3 inhibits IL-12 but not TNF-α [18]. IL-12 promotes Pg killing[18], whereas TNF-α induces destructive inflammation [9].
3	Tissue destruction is predominantly caused by endogenous inducers of inflammation.	Breakdown products of extracellular matrix are potent inflammatory stimuli [40], and Pg degrades extracellular matrix [15].
4	Janus-like doubled-faced character of the Pg species represented by distinct strains: **a.** Type I FimA strains may be stealthy. **b.** Type II or IV FimA strains may be overtly aggressive.	Pg *fimA* displays genetic diversity which determines, to a large degree, proinflammatory and virulence properties of fimbrial genotypes [44,45]. Numerous Pg mobile genetic elements appear to generate a plethora of strain-specific protein-coding sequences [49]. Virulence diversity may work in synergy to promote the survival of the Pg species.

* Does not fully explain the mechanism but involves documented examples which offer plausibility.