MINIREVIEW

Porphyromonas gingivalis: an invasive and evasive opportunistic oral pathogen

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Abstract
Porphyromonas gingivalis is a Gram-negative oral anaerobe that is involved in the pathogenesis of periodontitis, an inflammatory disease that destroys the tissues supporting the tooth, eventually leading to tooth loss. Porphyromonas gingivalis has the capacity to invade periodontal tissues and evade the host defence mechanisms. In doing so, it utilizes a panel of virulence factors that cause deregulation of the innate immune and inflammatory responses. The present review discusses the invasive and evasive strategies of P. gingivalis and the role of its major virulence factors in these, namely lipopolysaccharide, capsule, gingipains and fimbriae. Moreover, the role of P. gingivalis as a ‘keystone’ biofilm species in orchestrating a host response, is highlighted.

Introduction
Porphyromonas gingivalis and association with periodontal disease

Periodontal disease, or periodontitis, is defined as a bacterially induced inflammatory disease of the tooth-supporting (periodontal) tissues. Although more than 700 bacterial species can colonize the oral cavity (Aas et al., 2005), only a handful of those are highly implicated in the disease (Paster et al., 2006). Porphyromonas gingivalis is the species most highly associated with the chronic form of periodontitis, and can be detected in up to 85% of the disease sites (Yang et al., 2004). It is detected rarely or at low in numbers in healthy sites. The presence of P. gingivalis in a periodontal pocket may predict imminent disease progression (van Winkelhoff et al., 2002) and a significant positive correlation is found between P. gingivalis numbers and pocket depth (Kawada et al., 2004). Following periodontal treatment, a reduction of P. gingivalis numbers is associated with resolution of disease at the affected site (Haffajee et al., 1997; Fujise et al., 2002). Moreover, experimental implantation of P. gingivalis in animal models induces an inflammatory response and periodontal bone loss (Evans et al., 1992; Hajishengallis et al., 2011). This species possesses a number of potential virulence factors, such as cysteine proteinases (gingipains), lipopolysaccharide (LPS), capsule and fimbriae (Lamont & Jenkinson, 1998). Collectively, due to these properties P. gingivalis is considered an ‘opportunistic pathogen’, in line with the modified Koch’s postulates for oral infections, such as periodontal diseases (Socransky, 1979).

Structural and growth characteristics of P. gingivalis

Porphyromonas gingivalis is a black-pigmented, assaccharolytic, non-motile Gram-negative species that requires anaerobic conditions for growth, and the presence of heme or hemin and vitamin K in its nutrient milieu. It gains its metabolic energy by fermenting amino acids, a
property decisive for its survival in deep periodontal pockets, where sugars are extremely scarce. When considering its location in multispecies subgingival biofilm communities, *P. gingivalis* is a late colonizer, and hence is found in close proximity to and interacts with the juxtaposing gingival tissue (Kolenbrander et al., 2011; Zijlstra et al., 2011). The black pigmentation of *P. gingivalis* colonies observed in blood agar culture is itself associated with the aggregation of heme on its cell surface (Liu et al., 2004; Smalley et al., 2006). This property is somehow connected to its capacity to act as an opportunistic pathogen, as when grown in a heme-limited medium it becomes less virulent (McKee et al., 1986).

**Invasion of the host by *P. gingivalis***

As part of its strategies for survival into the host, *P. gingivalis* is able to invade cells and tissues (Yilmaz, 2008), thus avoiding the immune surveillance. *Porphyromonas gingivalis* can actively invade gingival epithelial cells, where it can maintain viability and replicate (Belton et al., 1999; Tribble et al., 2006). This invasive property is dependent on its major fimbriae, which bind to β1 integrin on the surface of host cells, an event that causes rearrangements of the actin cytoskeleton to allow intercellular migration (Yilmaz et al., 2002, 2003). *Porphyromonas gingivalis* can also invade macrophages, but within these cells its replication is less active (Wang et al., 2007). This is potentially a strategy for limited exposure to the extracellular environment and evasion of the immune surveillance. Interestingly, once *P. gingivalis* has invaded intracellularly, there are no signs of apoptosis or necrosis (Nakhjiri et al., 2001). It can then actively secrete an ATP-hydrolysing enzyme, thus suppressing ATP-dependent apoptosis (Yilmaz et al., 2008) and allowing its survival in host cells. Subsequently, it can disseminate from cell to cell, through actin cytoskeleton bridges without causing cell death, and spread while avoiding immune surveillance (Yilmaz et al., 2006). Once *P. gingivalis* is established in the cell, it affects cell-cycle pathways and thus accelerates proliferation of gingival epithelial cells, in a fimbriae-dependent fashion (Kuboniwa et al., 2008). This could well constitute a mechanism of expansion of the periodontal pocket epithelium, which is a histopathological feature of periodontitis.

**Survival strategies of *P. gingivalis***

It is now well established that *P. gingivalis* is not an aggressor of the inflammatory response, but rather an opportunist that can cross-talk with the host and subvert its defence mechanisms. Using this strategy, *P. gingivalis* prolongs its survival and becomes established in the periodontal pocket (Hajishengallis et al., 2011). It preferentially deregulates innate immunity, which may in turn disable adaptive immunity (Hajishengallis, 2009; Pathirana et al., 2010). Important representative examples of these abilities are its capacity to degrade human defensins (Carlisle et al., 2009), its resistance to oxidative burst-killing by polymorphonuclear neutrophils (PMNs) (Mydel et al., 2006) and its ability to inhibit ‘at will’ the production of crucial proinflammatory cytokines (Bostanci et al., 2007a, b). Although *P. gingivalis* has the capacity to stimulate interleukin (IL)-8 production by epithelial cells (Sandros et al., 2000; Asai et al., 2001; Kusumoto et al., 2004), it can also inhibit IL-8 production, resulting in hindered PMN chemotaxis, a phenomenon known as ‘chemokine paralysis’ (Darveau et al., 1998). *Porphyromonas gingivalis* thereby incapacitates the first line of defence in the periodontal tissues. Moreover, by inhibiting IL-12 production by macrophages, it prevents cytotoxic T-cell activation and therefore bacterial clearance (Hajishengallis et al., 2007). Accordingly, by inhibiting interferon (IFN)-γ production by T cells, it inhibits macrophage bacteriocidal activity and hence bacterial clearance (Pulendran et al., 2001; Hajishengallis et al., 2007). A special relationship is also revealed between *P. gingivalis* and the complement system, as it can suppress its activation, that is by degradation of C3 and capturing of C4b-binging protein, but also by synergizing with C5a via exploiting toll-like receptor (TLR)-2 signalling (Wang et al., 2010). A further interesting point is that whole viable *P. gingivalis* is differentially sensed by the host, compared with its released virulence factors, with the potential to activate distinctive intracellular pathways (Pathirana et al., 2010), or differential cytokine production (Zhou et al., 2005).

**Virulence factors of *P. gingivalis***

As an opportunistic pathogen, it is not surprising that *P. gingivalis* possesses a number of virulence factors. These are molecules that can elicit deleterious effects on host cells, essentially the survival ‘weapons’ of *P. gingivalis*. The main virulence factors discussed here are LPS, capsular polysaccharide (CPS), fimbriae and gingipains.

**The LPS of *P. gingivalis***

Like all Gram-negative bacterial species, *P. gingivalis* is sheathed by an LPS, which is an outer membrane component recognized by the host that can trigger intracellular signalling events. The affinity of LPS to its pattern recognition receptors, such as the TLRs and CD14, enables discrimination between commensal and pathogenic species. The *P. gingivalis* LPS is a stimulator of proinflammatory
responses and bone resorption, as demonstrated in experimental animal models (Chiang et al., 1999; Nishida et al., 2001). In vitro, it stimulates proinflammatory cytokine production of, for example, IL-1α, IL-1β, IL-6, IL-8, IL-18 and tumour necrosis factor (TNF)-α in monocytes (Zhou et al., 2005; Bostanci et al., 2007a, b; Hamedi et al., 2009). Yet, P. gingivalis LPS exhibits controversial features with regard to the induction of an inflammatory response. Apart from being a weaker cytokine stimulator compared with the LPS of other Gram-negative (i.e. enteropathogenic) species (Liu et al., 2007), it can also antagonize the cytokine-stimulating capacity of other putative pathogens (Bostanci et al., 2007a, b).

Structurally, P. gingivalis LPS exhibits unique features compared with the LPS of other species. These include differences in the structure of the O-antigen between P. gingivalis strains that can confer antigenic differences (Paramonov et al., 2001, 2009), as well as in the acylation patterns and receptor-activating capacities of the lipid A component. While the lipid A of most Gram-negative species is a strong activator of TLR4 responses, P. gingivalis lipid A is predominantly a TLR2 activator and may even act as antagonist to TLR4 (Darveau et al., 2004), dampening the immune responses (Hajishengallis, 2009). When considering further the heterogeneous acylation patterns of P. gingivalis lipid A, two forms are predominant: the tetra-acylated and penta-acylated forms. These two structures induce opposing host responses. The penta-acylated lipid A activates TLR4, whereas tetra-acylated lipid A acts as a TLR4 antagonist (Darveau et al., 2004; Nemoto et al., 2006). These changes of P. gingivalis lipid A acylation are dependent on microenvironmental conditions. In particular, when hemin availability is high (a condition that reflects inflammation), penta-acylated lipid A is converted into tetra-acylated lipid A (Al-Qutub et al., 2006). Hence, by modifying its lipid A structure according to the microenvironment, P. gingivalis may modulate the binding affinity of its LPS to its cognate TLR receptors, subsequently selecting how to affect downstream host immune signalling. Interestingly, a second type of LPS has also been identified in P. gingivalis, containing a distinct anionic polysaccharide linked to lipid A, known as A-LPS (Paramonov et al., 2005). A-LPS is required for cell integrity and serum resistance (Shoji et al., 2002; Paramonov et al., 2005; Slaney et al., 2006) and is structurally associated with the Arg-X gingipain (Curtis et al., 1999; Paramonov et al., 2005). It is also a weaker inducer of cytokine responses by human monocytes, as compared with the conventional LPS (Rangarajan et al., 2008). Collectively, the modifications and heterogeneity of P. gingivalis LPS can result in opposing actions and immunological deregulation. Strategically, this is in line with the manipulation of host innate immune responses by this species, to facilitate its adaptation and survival into the host.

The CPS of P. gingivalis

A major virulence factor of P. gingivalis is considered to be its capsule, also known as CPS or K-antigen (Schifferle et al., 1989; Holt et al., 1999; Farquharson et al., 2000; Aduse-Opoku et al., 2006; Brunner et al., 2010a, b). Based on the capacity of CPS to generate systemic IgG antibody responses, at least six different serotypes have been identified (Laine et al., 1997; Sims et al., 2001). Encapsulated P. gingivalis strains are shown to be highly invasive, causing spreading infection in a murine lesion model, whereas nonencapsulated strains induced only localized abscesses (Laine & van Winkelhoff, 1998). Interestingly, immunization with P. gingivalis CPS induced a high IgG systemic response (Choi et al., 1998) and reduced P. gingivalis-induced alveolar bone loss (Gonzalez et al., 2003). Encapsulated strains of P. gingivalis are more resistant to phagocytosis by polymorphonuclear leukocytes than nonencapsulated strains (Sundqvist et al., 1991) and have differential capacities to adhere to gingival epithelial cells (Dierickx et al., 2003). Moreover, differences in CPS serotypes can reflect differential capacities in chemokine stimulation by macrophages (d’Empaire et al., 2006) or cytokine stimulation by dendritic cells (Vernal et al., 2009). Interestingly, a nonencapsulated P. gingivalis knockout mutant strain was found to be a more potent inducer of cytokine synthesis by human gingival fibroblasts, as compared with the corresponding wild-type strain, implying a role of CPS in downplaying the innate immune responses (Brunner et al., 2010a, b). Although it is evident that the presence of CPS, or its individual serotypes, could be determinants of the virulence of P. gingivalis, the potential involvement of this antigen in the overall deregulation of host responses awaits further clarification.

The fimbriae of P. gingivalis

The fimbriae of P. gingivalis are thin, filamentous cell-surface protrusions that facilitate its adherence to salivary proteins, extracellular matrix, eukaryotic cells and bacteria of either the same or other species. Through its fimbriae, P. gingivalis can thus attach to early colonizing bacteria, and participate in the developing biofilm structure. Type I (major) fimbriae have important roles in colonization and invasion, whereas type II (minor) fimbriae possess a higher proinflammatory capacity (Lamont & Jenkinson, 1998; Amano et al., 2004; Hajishengallis et al., 2008). Interestingly, however, P. gingivalis strains W50 and W83 that lack major fimbriae are still invasive, as...
The gingipains of *P. gingivalis*

Gingipains are a group of cell surface cysteine proteinases of *P. gingivalis* that can also be present in secreted soluble form. They account for 85% of the total proteolytic activity of *P. gingivalis* (Potempa *et al.*, 1997). Based on their substrate specificity, they are divided into arginine-specific (Arg-X) and lysine-specific (Lys-X) gingipains (Curtis *et al.*, 2001; Guo *et al.*, 2010). Arg-X gingipains have trypsin-like activity, and can degrade extracellular matrix components, including the integrin–fibronectin-binding, cytokine, immunoglobulin and complement factors. There are two types of Arg-X gingipains, namely RgpA, which contains a proteolytic and an adhesion domain, and RgpB, which contains only the proteolytic domain. There is one type of Lys-X gingipain, Kgp, which contains both a proteolytic and an adhesion domain. There are sequence similarities between the adhesion domains of Kgp and RgpA (Curtis *et al.*, 2001).

The gingipains have multiple effects on the molecular components of the immune response, and as such they can deregulate these responses. For instance, they can cleave several T-cell receptors, such as CD2, CD4 and CD8 (Kitamura *et al.*, 2002), thereby hampering the cell-mediated immune response. They can also stimulate expression of protease-activated receptors in neutrophils (Lourbakos *et al.*, 1998), gingival epithelial cells (Lourbakos *et al.*, 2001), gingival fibroblasts and T cells (Belibasakis *et al.*, 2010), which are crucial for the induction of cytokine responses and the establishment of chronic inflammation in periodontitis (Holzhausen *et al.*, 2010; Fagundes *et al.*, 2011). Gingipains can also stimulate IL-6 production by oral epithelial cells (Lourbakos *et al.*, 2001) and IL-8 production by gingival fibroblasts (Oidomori *et al.*, 2001), enhancing the inflammatory responses. However, they can also proteolytically inactivate both anti-inflammatory (IL-4, IL-5) and pro-inflammatory (IL-12, IFN-γ) cytokines (Yun *et al.*, 1999, 2001, 2002; Tam *et al.*, 2009).

A number of particularly interesting effects are exerted by the gingipains on components of the complement system. Arg-X gingipains can cleave the C5 molecule, resulting in release of its C5a component, which is crucial for enhancing the recruitment of PMNs (Wingrove *et al.*, 1992; Imamura *et al.*, 2001). On the other hand, Lys-X can inactivate the C5a receptor on PMNs, an action that may actually impair their recruitment (Jagels *et al.*, 1996a, b). Along this line, the Arg-X gingipains can degrade the C3 molecule, potentially contributing to decreased bacterial opsonization (Schenkein *et al.*, 1995). This property could confer increased resistance of *P. gingivalis* to bactericidal activity.

Apart from their effect on immune responses, gingipains may also be involved in the binding of *P. gingivalis* to host cells, as Rgp–Kgp complexes have been shown to mediate adherence on gingival epithelial cells and gingival fibroblasts (Chen *et al.*, 2001; Grenier *et al.*, 2003; Andrian *et al.*, 2004). Interestingly, when *P. gingivalis* intracellularly invades gingival epithelial cells, expression of gingipain is downregulated (Xia *et al.*, 2007).

Gingipains may also affect vascular permeability and bleeding at the periodontal site. They can proteolytically activate plasma kallikrein and bradykinin, or alternatively increase the release of thrombin and prothrombin, which can result in increased vascular permeability and PMN influx (Imamura *et al.*, 1994, 1995a). Moreover, by degrading fibrinogen (Scott *et al.*, 1993), they may contribute to inhibition of blood coagulation and increase bleeding at the site (Imamura *et al.*, 1995b), thus enhancing the availability of hemin required for *P. gingivalis* growth.

Collectively, studies in various experimental systems indicate that gingipains have seemingly contradicting
actions on the innate immune responses, hampering interpretation of their role in the pathogenesis of periodontitis. Nevertheless, such differences may be reconciled by the existence of a concentration gradient of gingipains in the tissue (Pathirana et al., 2010). Closer to the gingival epithelial barrier where the biofilm resides, gingipain concentrations are high, causing degradation or deregulation of various components of the immune response. This hampers bacterial clearance and facilitates bacterial invasion. Nevertheless, deeper into the gingival connective tissue, gingipain concentrations become gradually lower and stimulate, rather than inhibit, inflammation. This may in turn induce connective tissue and bone destruction, which are the hallmarks of periodontitis.

Conclusions

It is evident that P. gingivalis has developed mechanisms to invade and persist into the host, by astutely adapting to its local niche. Its paradoxically opposing (stimulatory vs. inhibiting) effects on innate immune and inflammatory responses aim to subvert host defence mechanisms, in order to facilitate its survival in the tissue (Hajishengallis, 2009; Hajishengallis & Lambris, 2011). The net effect of this deregulated equilibrium is likely to determine if site-specific disease progresses beyond or remains at stationary phase. Whether inflammation is beneficial or deleterious to P. gingivalis may depend on the stage of its establishment in the host (Hajishengallis, 2009; Pathirana et al., 2010). At early stages, suppression of inflammation and evasion of host recognition would aid P. gingivalis in colonizing, invading and establishing at the targeted site. At later stages, once P. gingivalis is well established, inducing inflammation may facilitate its increased demands in nutrients. Alternatively, P. gingivalis may induce a ‘non-productive inflammation’, one that fails to eliminate it, yet is sufficient to induce mediators of tissue destruction (Hajishengallis, 2009).

Finally, as periodontitis is of polymicrobial nature, it is reasonable to consider the role of different bacterial species within the context of (sub)gingival biofilm communities. Hence, P. gingivalis is likely to function in concerted action with other species, to their mutual benefit. For instance, complement manipulation by P. gingivalis may denote a coevolution strategy to support other species present in the biofilm, which may reciprocally provide further colonization opportunities and nutrient availability to P. gingivalis. Subsequent changes in the local microenvironment can differentially regulate expression of its virulence factors, and hence the proinflammatory or anti-inflammatory potentials of P. gingivalis. This is strongly indicated by recent evidence demonstrating that even at low abundance, this species qualitatively and quantitatively affects the composition of the oral commensal microbiota, which are in turn required for P. gingivalis-induced inflammatory bone loss (Hajishengallis et al., 2011). For these reasons, P. gingivalis is now considered a ‘keystone’ species in subgingival biofilms (Honda, 2011).

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References


Porphyromonas gingivalis and host response


