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INVITED REVIEW

Beyond the red complex and into more complexity: the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology

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SUMMARY

Recent advancements in the periodontal research field are consistent with a new model of pathogenesis according to which periodontitis is initiated by a synergistic and dysbiotic microbial community rather than by select 'periopathogens', such as the 'red complex'. In this polymicrobial synergy, different members or specific gene combinations within the community fulfill distinct roles that converge to shape and stabilize a disease-provoking microbiota. One of the core requirements for a potentially pathogenic community to arise involves the capacity of certain species, termed 'keystone pathogens', to modulate the host response in ways that impair immune surveillance and tip the balance from homeostasis to dysbiosis. Keystone pathogens also elevate the virulence of the entire microbial community through interactive communication with accessory pathogens. Other important core functions for pathogenicity require the expression of diverse molecules (e.g. appropriate adhesins, cognate receptors, proteolytic enzymes and proinflammatory surface structures/ligands), which in combination act as community virulence factors to nutritionally sustain a heterotypic, compatible and proinflammatory microbial community that elicits a non-resolving and tissue-destructive host response. On the basis of the fundamental concepts underlying this model of periodontal pathogenesis, that is, polymicrobial synergy and dysbiosis, we term it the PSD model.

THE 'RED COMPLEX' AND THE MICROBIAL ETIOLOGY OF PERIODONTITIS

There is an old story of a woman out walking when she encounters a boy with his dog. 'That's a nice dog', says the woman, 'what's his name?' 'We call him Rover' the boy replies, 'but we don't know his real name'. The notion that individual organisms or even species may have properties that elude our classification systems is also applicable to bacteria, which may have inherent characteristics that are independent of our attempts to label them as 'pathogens' or 'commensals'. Indeed, the distinction between pathogens and commensals is becoming increasingly blurred, especially in diseases that ensue from the

action of bacteria that are also present in health and that involve complex host–microbe interactions. Such a case, par excellence, is human periodontal disease. Not surprisingly, therefore, periodontitis has a long and rich history of proposed microbial etiologies ranging from the 'non-specific plaque hypothesis' to implication of specific and varied microorganisms, including the oral protozoan *Entamoeba gingivalis* proposed a century ago (see Table 1) (Socransky & Haffajee, 1994; Wade, 2011).

Much of our current appreciation of the microbial etiology of periodontitis derives from detailed cultural characterization of the periodontal microbiota in the late 1970s and early 1980s. These studies revealed dramatic compositional changes to the microbiota in disease as compared with health (Slots, 1977a,b; Socransky, 1977; Tanner *et al.*, 1979; Moore *et al.*, 1982, 1983). One plausible interpretation was that these key findings pointed to bacterial specificity in the etiology of periodontitis, in that the disease-associated microbiota contained novel pathogenic species that were either absent or hardly detectable in health. The quest to identify specific periodontal pathogens led to significant progress, including the identification of a number of candidates and the characterization of putative virulence factors thereof (Socransky *et al.*, 1998; Holt & Ebersole, 2005). Using whole genomic DNA probes and checkerboard DNA–DNA hybridization, Socransky and colleagues characterized periodontal microbial communities on the basis of a color-coded system that reflected cluster analysis, community ordination and associated disease severity (Socransky *et al.*, 1998). Foremost among these groups was the so-called 'red complex', a group of three species including *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*, the detection of which was strongly associated with each other and with diseased sites (Socransky *et al.*, 1998).

Partly because *P. gingivalis* was the easiest of the red complex bacteria to grow and genetically manipulate, it became the most widely studied periodontal bacterium. Virulence of the organism was attributed to an array of molecules, including colonization factors (fimbriae and hemagglutinins) and functionally versatile proteolytic enzymes (gingipains) (Lamont & Jenkinson, 1998). In a landmark study, *P. gingivalis* was shown to cause periodontitis in non-human primates upon its oral implantation, which was interpreted as evidence for a specific microbial etiology in periodontitis (Holt *et al.*, 1988). More recently, longitudinal human studies demonstrated that the progression of chronic periodontitis can be predicted by the levels of *P. gingivalis* and *T. denticola* in subgingival plaque (Byrne *et al.*, 2009). The implication of specific periodontal bacteria as putative etiologic agents provided rationale and impetus for experimental vaccination against periodontal disease (Persson, 2005). Additional work focused primarily on understanding cellular and molecular pathogenic mechanisms of the red complex and certain other suspected pathogens (Cutler *et al.*, 1995; Sela, 2001; Holt & Ebersole, 2005; Sharma, 2010). As a note of caution, however, most of this research was performed in the context of a conventional host–pathogen interaction, as exemplified by diseases with defined infective etiology, and the extent to which these virulence factors are operational *in vivo* remains to be determined.

The convenience and appeal of the concept of red, and other color-coded, complex(es) led to widespread adoption up to the present day. However, as molecular-based approaches to microbe detection became increasingly facile, and as studies using culture-independent methodology for analysis of the periodontal microbiota became abundant, two newer concepts emerged. First, red complex organisms such as *P. gingivalis* can be found in the absence of disease, further argument against the organisms as classical exogenous pathogens (Ximenez-Fyvie *et al.*, 2000a, b; Mayanagi *et al.*, 2004; Diaz *et al.*, 2006). Second, the periodontal microbiota is more heterogeneous and diverse than previously thought (Dewhirst *et al.*, 2010; Curtis *et al.*, 2011b; Griffen *et al.*, 2011), with over 700 organisms recognized as possible components, of which around 200 can be present in any one individual and about 50 are present at any one site (Aas *et al.*, 2005). Many of these newly recognized organisms show as good or better a correlation with disease as the classical red complex (Kumar *et al.*, 2005, 2006; Griffen *et al.*, 2012). Novel disease-associated species include the gram-positive *Filifactor alocis* and *Peptostreptococcus stomatis* and other species from the genera *Prevotella*, *Megasphaera*, *Selenomonas*, *Desulfobulbus*, *Dialister* and *Synergistetes* (Paster *et al.*, 2001; Kumar *et al.*, 2003, 2005; Dewhirst *et al.*, 2010; Griffen *et al.*, 2011, 2012). Moreover, contrary to the 'dogma' of gram-negative bacterial dominance in periodontitis,

Table 1 Milestones and hypotheses in the microbial etiology of periodontitis[1]

Chronology	Etiology or key concepts	Remarks	References
Late 19th to early 20th century	Specific microorganisms (amebae, spirochetes, fusiforms or streptococci)	Based on apparent association with periodontal lesions; heavily biased by technique used (wet-mount microscopy, stained smear microscopy, and limited cultural methods)	Meyer (1917)
Mid-1920s to 1930s	Decline in the interest in bacteria as primary agents in the etiology of periodontitis	Disease primarily due to defects in the patient (e.g. trauma from occlusion); bacteria are simply secondary invaders or at best contributors to the disease process	Bruske (1928) and Belding & Belding (1933)
Late 1950s	'Non-specific plaque hypothesis' and its variant, 'Mixed anaerobic infections'	Sufficient accumulation of any microorganisms at or below the gingival margin can cause destructive inflammation through the local production of 'irritants' Bacteriologically non-specific but biochemically specific mixed anaerobic infections are capable of producing destructive metabolites	Schultz-Haudt *et al.* (1954) and Macdonald *et al.* (1956)
Late 1970s to early 1980s	Microbial shift in periodontitis	Dramatic compositional changes to the microbiota in disease as compared with health	Slots (1977a,b), Socransky (1977), Tanner *et al.* (1979) and Moore *et al.* (1982, 1983)
	'Specific plaque hypothesis'	Periodontitis results, at least in significant part, from the overgrowth of specific pathogenic species	Loesche (1979, 1992)
Late 1980s to 1990s	'Red complex'	Periodontal microbial communities characterized on the basis of a color-coded system reflecting cluster analysis, community ordination, and associated disease severity. A group of 'red complex' bacteria (*Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*) are strongly associated with each other and with diseased sites. *Porphyromonas gingivalis* causes periodontitis in nonhuman primates upon its oral implantation	Holt *et al.* (1988) and Socransky *et al.* (1998)
2003	'Ecological catastrophe hypothesis'	Environmental factors drive the selection and enrichment of specific pathogenic bacteria	Marsh (2003)
2010	Disruption of periodontal tissue homeostasis	Periodontitis fundamentally represents disruption of tissue homeostasis; inflammation is critical but a secondary event. Red complex bacteria are key species for disease, although the polymicrobial nature of periodontitis is acknowledged; commensal bacteria probably induce a protective response	Darveau (2010)
2011	'Keystone pathogen' concept	Low-abundance keystone species can disrupt tissue homeostasis through quantitative and qualitative changes to the commensal microbiota (resulting, at least in great part, from host modulation by the keystone pathogen). Inflammatory bone loss is mediated by the altered microbiota	Hajishengallis *et al.* (2011)
2012	'Polymicrobial synergy and dysbiosis' (PSD)	Periodontitis is initiated by a synergistic and dysbiotic microbiota, within which different members, or specific gene combinations thereof, fulfill distinct roles that converge to shape and stabilize a disease-provoking microbiota. Combines the concepts of 'disrupted homeostasis' and 'keystone pathogen' but questions the primary importance of the red complex	This paper

[1]For a detailed historic description of the various hypotheses, the reader is referred to Socransky & Haffajee (1994).

gram-positive anaerobic species exhibit a significant increase in deep diseased sites relative to healthy sites, and can be detected in greater abundance than gram-negative species in some studies (Kumar *et al.*, 2005). Many of these species are as yet to be cultivated, however, study of the more tractable organisms is underway and *F. alocis*, for example, has the potential to withstand oxidative stress (Aruni *et al.*, 2011) and to induce strong proinflammatory responses (Moffatt *et al.*, 2011).

Although the pathogenic credentials of most of these newly recognized organisms remain to be established, it is evident that the concept of a three-species red complex as representing the primary etiologic unit in periodontitis requires refinement. Not only are there more potential pathogens to consider, but these organisms can exhibit polymicrobial synergy (discussed in more detail below). Furthermore, a disease-associated shift in the composition of the periodontal microbiota could have alternative explanations. The findings could be interpreted to imply that periodontitis is caused by a dysbiotic change in the periodontal microbiota, that is, a change in the relative abundance of individual members of the microbiota relative to their abundance in health. The altered microbiota could in turn lead to changes in the host–microbe crosstalk sufficient to initiate chronic, non-resolving inflammatory disease. Disease can then be seen in terms of an ecological catastrophe as defined by Marsh (2003). In the following text we shall attempt to integrate these concepts into a new model of periodontitis that accommodates the data derived from sequencing projects, the interactions that occur among organisms and their differential pathogenicity (Fig. 1).

THE *PORPHYROMONAS GINGIVALIS* PARADOX

To understand basic principles of periodontal microbial pathogenicity, it is instructive to first consider the case of *P. gingivalis*. This organism can cause alveolar bone loss in animal models of periodontal disease, although, as we shall see, pathogenicity is context dependent. The low abundance of *P. gingivalis* in periodontitis-associated dental plaque was noted in early bacteriological studies (Moore *et al.*, 1982). This, together with findings that *P. gingivalis* is not a potent inducer of inflammation by itself, presented an

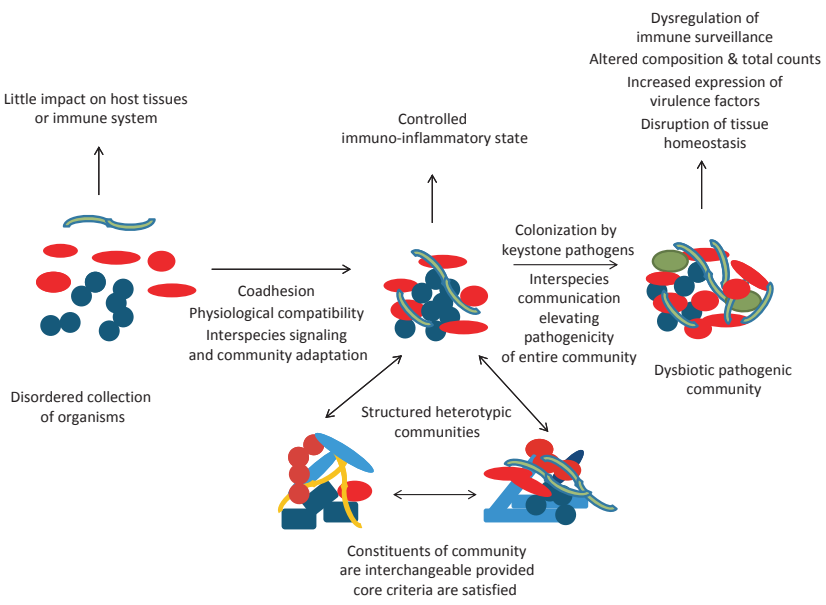

Figure 1 The polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. The gingival crevice is colonized by a diverse microbiota, and compatible organisms assemble into heterotypic communities. These communities are in equilibrium with the host. Although they are proinflammatory and can produce toxic products such as proteases, overgrowth and overt pathogenicity are controlled by the host. The microbial constituents of the communities can vary over time, and from person to person and site to site. Colonization by keystone pathogens such as *Porphyromonas gingivalis* elevates the virulence of the entire community following interactive communication with accessory pathogens such as mitis group streptococci. Host immune surveillance is impaired and the dysbiotic community increases in number eventually disrupting tissue homeostasis and causing destruction of periodontal tissues.

apparent paradox that could not be readily reconciled with a proactive role in an inflammatory disease. For instance, *P. gingivalis* expresses an atypical lipopolysaccharide with a 4-acyl monophosphorylated lipid A moiety that can potently antagonize Toll-like receptor 4, unlike the highly proinflammatory lipopolysaccharides of most other gram-negative bacteria (Coats *et al.*, 2009; Darveau, 2010). *Porphyromonas gingivalis* can invade and survive within gingival epithelial cells; however, rather than causing host cell damage, internalized bacteria induce an anti-apoptotic, pro-survival phenotype in host cells (Mao *et al.*, 2007; Kuboniwa *et al.*, 2008) and downregulate expression of bacterial proinflammatory components such as fimbrial proteins and proteases (Xia *et al.*, 2007; Hendrickson *et al.*, 2009). Moreover, in contrast to other periodontal bacteria that stimulate interleukin-8 release from gingival epithelial cells, *P. gingivalis* actually inhibits the production of this proinflammatory chemokine by means of a secreted serine phosphatase (SerB) (Darveau *et al.*, 1998; Hasegawa *et al.*, 2008). The release of interleukin-8 by the junctional gingival epithelium is regarded as an important feature of the healthy periodontium because it generates a gradient for neutrophil recruitment into the gingival crevice (Tonetti *et al.*, 1998; Darveau, 2010).

A recent study in mice has offered an explanation for this apparent paradox and proposed a disease model that is consistent with the alternative interpretation of the periodontitis-associated microbial shift. This study showed that *P. gingivalis* can impair innate immunity in ways that enhance the growth and alter the composition of the periodontal microbiota (Hajishengallis *et al.*, 2011). Specifically, *P. gingivalis*, at low colonization levels (<0.01% of the total microbiota), remodeled a symbiotic community into a dysbiotic state that triggered inflammatory bone loss. *Porphyromonas gingivalis* failed to cause periodontitis in the absence of commensal bacteria, i.e. in germ-free mice, despite its ability to colonize this host (Hajishengallis *et al.*, 2011). The capacity of *P. gingivalis* to exert a community-wide impact that tipped the balance towards dysbiosis, while being a quantitatively minor constituent of this microbial community, has prompted its characterization as a keystone pathogen, in analogy to the crucial role of a single keystone in an arch (Hajishengallis *et al.*, 2011).

The keystone effects of *P. gingivalis* are likely exerted via both host modulation and bacterial synergy (discussed below). In terms of host modulation, *P. gingivalis* can transiently inhibit the induction of gingival interleukin-8-like chemokines, which could delay the recruitment of neutrophils and, thereby, facilitate its initial colonization and promote the fitness of other organisms (Hajishengallis *et al.*, 2011). However, the ability of *P. gingivalis* to persist in the periodontium appears to depend on the complement C5 convertase-like activity of its gingipains and the instigation of a subversive crosstalk between C5a receptor (C5aR) and Toll-like receptor 2 (Hajishengallis *et al.*, 2011), earlier shown to impede the killing capacity of leukocytes (Wang *et al.*, 2010; Liang *et al.*, 2011). Consistent with this, *P. gingivalis* fails to cause dysbiosis and periodontitis in mice lacking C5aR.

Although established in the mouse model, the keystone pathogen concept is consistent with observations in other animal models and in humans (reviewed by Darveau *et al.*, 2012). Briefly, in rabbits, *P. gingivalis* causes a shift to a more anaerobic microbiota and an overall increase in the bacterial load of the dental biofilm (Hasturk *et al.*, 2007). In non-human primates (*Macaca fascicularis*), where *P. gingivalis* is a natural inhabitant of the periodontal biofilm, a gingipain-based vaccine causes a reduction in *P. gingivalis* numbers and in the total subgingival bacterial load (Page *et al.*, 2007), suggesting that the presence of *P. gingivalis* benefits the entire community. A significant increase in the total microbial load is also observed in human periodontitis compared with health (Socransky & Haffajee, 1994; Darveau *et al.*, 1997); however, it has not been yet addressed whether this is causally linked to the presence of *P. gingivalis* or other bacteria acting as keystone pathogens. Nevertheless, consistent with a keystone pathogen role, *P. gingivalis* is a quantitatively minor constituent of periodontitis-associated biofilms (Moore *et al.*, 1982; Doungudomdacha *et al.*, 2000; Kumar *et al.*, 2006), despite its high prevalence and association with progressive bone loss in periodontitis patients (Moore *et al.*, 1991; Chaves *et al.*, 2000).

The subversion of recruited leukocytes by *P. gingivalis* likely facilitates uncontrolled growth of other species in the same biofilm. This disruption of tissue homeostasis, in turn may allow other community members to trigger destructive inflammation. Such a host response, apart from its adverse impact on the integrity of the periodontium, leads to tissue

breakdown products that serve the nutritional needs of the community at large (Gaffen & Hajishengallis, 2008). Consequently, the transition to a disease-provoking microbiota may be stabilized and, conversely, those species that cannot thrive under inflammatory environmental conditions, or for which host inflammation is detrimental, may be outcompeted or eliminated. It should be clarified, however, that disruption of tissue homeostasis followed by dysbiosis can also be caused by host regulatory defects in the absence of a keystone pathogen. For instance, genetic deficiency of Del-1, an endothelial cell-derived protein that regulates neutrophil recruitment, causes inflammatory bone loss and both quantitative and compositional alterations to the murine commensal microbiota in the absence of *P. gingivalis* (Eskan *et al.*, 2012). This implies that, at least in principle, periodontitis could be initiated in the absence of bacteria belonging to the 'red complex' or acting as keystone pathogens.

As mentioned, *P. gingivalis* can also be detected, albeit less frequently, in periodontally healthy individuals (Haffajee *et al.*, 1998; Ximenez-Fyvie *et al.*, 2000a,b; Mayanagi *et al.*, 2004; Diaz *et al.*, 2006), which begs the question why its presence does not always lead to periodontitis. In other words, why is the organism a pathogen in some instances and a commensal in others. Several not mutually exclusive explanations may involve variability in the status of the host or the bacterium. There may be individuals who can either resist or tolerate the conversion of the periodontal microbiota from a symbiotic to a dysbiotic state, by virtue of their intrinsic immuno-inflammatory status (e.g. hyporesponsive or lack-of-function polymorphisms that attenuate inflammation or microbial immune subversion). Moreover, strain and virulence diversity within the population structure of *P. gingivalis* may affect its capacity to act as a keystone pathogen. Additionally, local environmental changes may influence the capacity of *P. gingivalis* to disrupt host–bacteria homeostasis. In this regard, the production of *P. gingivalis* gingipains and fimbriae is regulated by local environmental conditions (Xie *et al.*, 1997, 2000; Curtis *et al.*, 2001), which can therefore influence the capacity of this organism to modulate complement activity and subvert leukocytes (Hajishengallis *et al.*, 2011). In the same vein, the structure of *P. gingivalis* lipopolysaccharide is regulated by temperature fluctuations and hemin concentrations (Al-Qutub *et al.*, 2006; Curtis *et al.*, 2011a). Therefore, at least theoretically, there are conditions under which *P. gingivalis* (or any other single bacterium whether from the 'red complex' or not) may not act as a pathogen but rather behave as a commensal.

THE REAL CULPRIT: A SYNERGISTIC MICROBIAL COMMUNITY

It is becoming evident, therefore, that the virulence of periodontal pathogens such as *P. gingivalis* acquires importance only in the context of a synergistic microbial community, which is required for the expression of pathogenicity. This model is consistent with the participation of both gram-negative and gram-positive bacteria in periodontal pathogenesis, as long as they can provoke or tolerate inflammation, or provide other useful service to the community. Mixed microbial communities provide opportunities for competitive and co-operative interspecies interactions, and such interactions shape the nature and function of the entire assemblage (Jenkinson & Lamont, 2005; Hansen *et al.*, 2007). Furthermore, interspecies signaling within communities provides the opportunity to collectively regulate activities including gene expression, nutrient acquisition and DNA exchange. In this manner communities of bacteria can exhibit polymicrobial synergy, defined as an increase in the ability of a bacterium to colonize/persist or elevate disease symptoms when in the presence of other bacteria (Fig. 1). Indeed, there are numerous examples of mixed infections with oral organisms exhibiting increased pathogenicity compared with either organism alone in animal models (Kesavalu *et al.*, 2007; Orth *et al.*, 2011; Settem *et al.*, 2012). To some degree this will reflect nutritional cross-feeding that enhances the growth rate of compatible organisms in combination (Grenier, 1992; Nilius *et al.*, 1993), and, moreover, closely associated organisms can compile a communal suite of enzymes to sequentially degrade complex substrates into constituents that can be metabolized by individual members of the community. However, as mentioned above, *P. gingivalis* can have a community-wide pathogenic influence on the microbiota in animals, at least in part via host modulation (Hajishengallis *et al.*, 2011). Moreover, the introduction of *P. gingivalis* into a healthy multispecies biofilm alters the pattern of microbial community gene expression (Frias-Lopez & Duran-Pinedo, 2012),

suggesting that this keystone pathogen could additionally modulate the commensal oral microbiota through direct, host-independent effects. Conversely, when in a community with *Streptococcus gordonii* and *Fusobacterium nucleatum*, *P. gingivalis* differentially expresses around 500 proteins, indicating that the organism undergoes profound phenotypic changes in response to common oral species (Kuboniwa *et al.*, 2009). Polymicrobial synergy among periodontal pathogens therefore extends beyond growth rate effects and involves interspecies signaling and response interactions.

The molecular mechanisms that underlie the polymicrobial synergy of oral bacteria reveal hierarchical, temporally distinct communication systems whereby organisms can integrate multiple signals of various forms and function during the process of heterotypic community development. Oral bacteria can communicate through contact-dependent systems, and short range diffusible signals, including metabolic products and autoinducer-2 (Kolenbrander *et al.*, 2002, 2010), all of which can influence pathogenic potential (Ramsey & Whiteley, 2009; Ramsey *et al.*, 2011). For example, *P. gingivalis* displays numerous specific interactions with oral streptococci, suggesting that interspecies co-operation has evolved to enhance the fitness of these organisms. Specific adherence of *P. gingivalis* to *S. gordonii* is mediated by the minor fimbrial subunit protein (Mfa1) which interacts with discrete domains on the streptococcal surface proteins SspA/B (Demuth *et al.*, 2001; Park *et al.*, 2005; Daep *et al.*, 2008). Coadhesion between the organisms initiates a signal transduction pathway within *P. gingivalis* based on protein tyrosine (de)phosphorylation that converges on regulation of genes encoding community effectors such as Mfa1 and LuxS (Maeda *et al.*, 2008; Chawla *et al.*, 2010). The level of community accumulation is tightly controlled possibly to maintain an optimal surface area : volume ratio of the dual species microcolony. The *P. gingivalis*–*S. gordonii* communities exhibit mutualistic growth (Periasamy & Kolenbrander, 2009) and induce greater alveolar bone loss in mice compared with either species alone (Daep *et al.*, 2011). Moreover, interference with *P. gingivalis*–*S. gordonii* binding by blocking the Mfa1 –SspA/B interaction abrogates bone loss, demonstrating the importance of coadhesion and subsequent signaling in the development of a pathogenic community (Daep *et al.*, 2011).

Communication relevant to virulence also occurs between the oral streptococci and *Aggregatibacter actinomycetemcomitans* which is closely associated with localized aggressive periodontitis. *Aggregatibacter actinomycetemcomitans* displays resource partitioning to favor carbon sources such as lactate generated by streptococcal metabolism (Brown & Whiteley, 2007). Additionally, *A. actinomycetemcomitans* can sense hydrogen peroxide, a metabolic byproduct of streptococci, and respond by upregulation of genes that enhance resistance to killing by neutrophils (Ramsey & Whiteley, 2009). The relevance of these interactions is demonstrated by co-culture of *A. actinomycetemcomitans* with *S. gordonii*, which enhances the pathogenicity of *A. actinomycetemcomitans* in a mouse abscess model (Ramsey *et al.*, 2011).

It is interesting to note that both of the above examples involve the mitis group oral streptococci, traditionally seen as commensals in the oral cavity. However, although the relative amounts decrease, oral streptococci still comprise a significant portion of the microbial population of subgingival plaque (Moore & Moore, 1994; Kroes *et al.*, 1999; Paster *et al.*, 2001; Kumar *et al.*, 2005; Quirynen *et al.*, 2005; Colombo *et al.*, 2009), and indeed some studies have shown a higher proportion of *S. gordonii* in the subgingival biofilm of periodontitis subjects compared with healthy individuals (Abiko *et al.*, 2010). It has been proposed, therefore, that organisms such as the mitis group streptococci be considered 'accessory pathogens', organisms whose pathogenic potential only becomes evident in the context of a heterotypic microbial community (Whitmore & Lamont, 2011). Periodontal pathogenicity would therefore appear to require significant interspecies co-operation.

THE PSD MODEL

The recent advancements discussed above are consistent with a new model of pathogenesis according to which periodontitis is initiated by a broadly-based dysbiotic, synergistic, microbiota (Fig. 1), as opposed to the traditional view of a conventional infectious disease caused by a single or even several select periopathogens, such as the 'red complex'. The situation can be likened, perhaps, to that of a crew team. All the oars need to be manned but the identities of individual crew members, provided they are capable of rowing, are not important for forward progression. Some

functions, however, such as cox, are more important for coordinating characteristics such as direction and speed. In the periodontal ecosystem diverse bacteria (or specific combinations of genes within the community) may be able to fulfill distinct roles that converge to form and stabilize a disease-provoking microbiota. Hence, there will be a number of core requirements for a potentially pathogenic community to arise. (i) Bacterial constituents will express the relevant adhesins and receptors to allow assembly of a heterotypic community. (ii) Individual members of the community will be physiologically compatible or at least non-antagonistic. (iii) The combined activities of the community will resist the host innate and acquired immune responses and contribute to tissue inflammation through, for example, proteolytic activity and cytokine induction. On the basis of the diversity of organisms associated with periodontal lesions, it is likely that potentially pathogenic communities occur frequently and that there are a variety of organisms that can contribute the genes necessary for these conditions to be satisfied. In that regard, it is relevant that dental plaque obtained from healthy sites shares the capacity of disease-associated plaque to induce strong inflammatory responses through Toll-like receptor activation (Yoshioka *et al.*, 2008). However, for pathogenic potential to be realized, the activities of a keystone species such as *P. gingivalis* are required. These organisms engage in two-way communication with the community inhabitants, in particular the accessory pathogens, to both disrupt host immune surveillance and elevate the pathogenicity of the entire group. This more specialized dysbiotic role will be restricted to fewer organisms. The identification of keystone and accessory pathogens from the catalog of organisms generated by microbiome projects will present the next major challenge in periodontal disease research and, perhaps, in other inflammatory diseases with a complex polymicrobial etiology (Hajishengallis *et al.*, 2012). Moreover, an in-depth understanding of periodontal pathogenesis on the basis of the PSD model may offer new targets for therapeutic intervention.

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