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Bacterial interactions in pathogenic subgingival plaque

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ABSTRACT

Chronic periodontitis has a polymicrobial biofilm aetiology. Polymicrobial biofilms are complex, dynamic microbial communities formed by two or more bacterial species that are important for the persistence and proliferation of participating microbes in the environment. Interspecies adherence, which often involves bacterial surface-associated molecules, and communications are essential in the spatial and temporal development of a polymicrobial biofilm, which in turn is necessary for the overall fitness of a well-organized multispecies biofilm community. In the oral cavity, interactions between key oral bacterial species, including *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*, are essential for the progression of chronic periodontitis. *In vivo, P. gingivalis* and *T. denticola* are frequently found to co-exist in deep periodontal pockets and have been co-localized to the superficial layers of subgingival plaque as microcolony blooms adjacent to the pocket epithelium, suggesting possible interbacterial interactions that contribute towards disease. The motility and chemotactic ability of *T. denticola*, although not considered as classic virulence factors, are likely to be important in the synergistic biofilm formation with *P. gingivalis*. *In vitro, P. gingivalis* and *T. denticola* display a symbiotic relationship in nutrient utilization and growth promotion. Together these data suggest there is an intimate relationship between these two species that has evolved to enhance their survival and virulence.

1. Introduction

The use of culture-dependent isolation techniques has previously characterized most diseases as monomicrobial, which means caused by a single species. The advancement of culture-independent community analyses using 16S rRNA and metagenomics sequencing methodologies acknowledged that some diseases, particularly chronic diseases, are polymicrobial in nature [1,2]. The oral cavity houses at least 500 species of microorganisms that live predominantly in biofilms [3]. Diseases of the teeth and gums, including caries and periodontal diseases, are associated with dental plaque, a polymicrobial biofilm accreted to the surface of the tooth. Chronic periodontitis is an inflammatory disease of the supporting tissues of the tooth, which results in destruction of those tissues, including the alveolar bone. If left untreated, the disease can ultimately lead to tooth loss. Chronic periodontitis has been regarded as a risk factor for cardiovascular diseases, preterm and underweight birth, diabetes, rheumatoid arthritis and certain cancers, such as those of the orogastrointestinal tract and pancreas [4–9]. Periodontitis is believed to be initiated by changes in the bacterial species composition of subgingival plaque and subsequent alteration of the host immune response [10]. Fifty years ago, chronic periodontitis was thought to progress in a slow, linear, continuously progressive manner. However, in 1984, based on the observations that the attachment loss rates are inconsistent with the continuous disease hypothesis and that rapid bursts of attachment loss were observed followed by periods of relative quiescence, Socransky et al. proposed a random burst model where chronic periodontitis was described to progress in recurrent acute episodes, with periods of activity alternating with intervals of remission [11]. Given the association of chronic periodontitis with the abundance and composition of bacteria in subgingival plaque, it could be hypothesized that chronic periodontitis progresses with repeated episodes of bacterial proliferation and dysbiosis followed by a reduction in the level of plaque through an effective host

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response and return to homeostasis. It has been proposed that pathogenic bacteria need to reach specific threshold levels in subgingival plaque before they can cause disease and that detection of these species above these thresholds may serve as predictors of periodontal disease progression. For example, the level of *Porphyromonas gingivalis* in subgingival plaque above threshold levels (~10% of total bacterial cell load) is predictive of imminent clinical attachment loss (disease progression) [10]. To fit this idea with the random burst model, one could hypothesize that the proliferation of specific bacterial species in the subgingival plaque above threshold levels causes a burst of destructive activity, which, at the same time, enhances host defense mechanisms that might eventually bring the levels of bacteria in the plaque back under the threshold level and result in a return to homeostasis and a period of quiescence. The bacteria in the plaque may then take advantage of the resolution of the host defense mechanism and start to proliferate again, leading to another burst of activity. The cumulative effects of these repeated disease episodes would ultimately lead to attachment loss. Several bacterial species are thought to be associated with the disease however *Treponema denticola*, *P. gingivalis* and *Tannerella forsythia* have together been categorized as a significant consortium named the "Red Complex" [12] due to their strong association with the clinical measurements of severe periodontal disease such as periodontal pocket depth and bleeding on probing [12–15]. *P. gingivalis* has more recently been shown to be a keystone pathogen that has the ability to dysregulate the host immune response and to disrupt host homeostasis, causing dysbiosis and polymicrobial biofilm proliferation [16].

2. Polymicrobial synergy

Polymicrobial synergy is a phenomenon where two or more bacterial species cooperatively interact to produce a result not achieved by the individual species [17]. It can occur between non-pathogenic species and thus the "result" does not necessarily refer to a disease outcome. In the context of infection Murray et al. [18] defined polymicrobial synergy as a phenomenon where the infection produced by two or more interacting microbes is more severe than that produced by the individual species. The synergistic virulence of *P. gingivalis*, *T. denticola* and *T. forsythia* in polymicrobial infection was evident through several studies using animal models of disease. A study using a murine abscess model showed that at low inocula of *P. gingivalis* where minimal or no lesions were induced with the bacterium alone, the addition of *T. denticola* significantly enhanced the formation of spreading lesions [19]. This is consistent with the idea that *T. denticola* facilitates the tissue invasion and damage mediated by *P. gingivalis* [15]. Another study using an alveolar bone loss rat model of periodontal disease demonstrated that intra-oral inoculation of *P. gingivalis*, *T. denticola* and *T. forsythia* at a 1:1:1 ratio (3.3×10^9 cells each) resulted in higher levels of bone resorption compared with mono-inoculations of each of the bacterial species (10^{10} cells) [20]. The synergistic virulence of *T. denticola* and *P. gingivalis* is also supported by a recent study using a murine experimental model of periodontitis, which showed a 40-fold reduction in total bacterial cells was needed to induce significant bone loss from co-inoculation of *P. gingivalis* and *T. denticola* as compared with inoculation of *P. gingivalis* alone [21]. The observed synergistic pathogenicity of *T. denticola* and *P. gingivalis* co-infections in animal models may be partly explained by the upregulation of *T. denticola* genes encoding virulence factors, such as dentilisin and major sheath protein (Msp), during co-culture of the two bacterial species [22], although it could also have resulted from enhanced colonization and persistence of the two bacteria at the infection site. Given the close proximity of each bacterial species in dental plaque, the synergistic pathogenesis is inevitably important in the progression of chronic periodontitis.

3. Polymicrobial biofilm formation

The majority of microbes form biofilms, attaching to biotic and abiotic surfaces, and rarely existing entirely in planktonic forms. Biofilm communities in most environments, including the human body, tend to be polymicrobial [23]. Polymicrobial biofilms are defined as "a varied collection of organisms (fungi, bacteria, and viruses) that exist at a phase or density interface and are coated in a self- and/or host-derived hydrated matrix, often consisting of polysaccharide" [24]. Several advantages are gained through the formation of a polymicrobial biofilm, such as passive resistance to antibiotics [25], metabolic cooperation [26,27], byproduct influence [28], quorum sensing systems [26], more efficient DNA sharing [29] and other synergies. During cohabitation and cocolonization, the microbes that form the biofilm community have evolved mutualistic or synergistic relationships or competitive antagonistic approaches that give them a competitive advantage over other microbes [2].

In the oral cavity where unattached bacteria are constantly removed by shear forces and bulk phase movement, formation of biofilm is essential for the persistence and proliferation of bacteria. The formation of polymicrobial biofilms involves several stages, whereby coaggregation or coadhesion is one of the essential steps to allow adherence and colonization of late colonizers to an established biofilm with antecedent bacteria. This subsequently increases the composition and number of participating microorganisms in the biofilm [30]. Coaggregation was observed among members of the oral microbiota and the process often involves bacterial surface proteins. *Streptococcus gordonii* is one of the early colonizers that initiate plaque formation and the ability of *P. gingivalis* to specifically recognize and interact with *S. gordonii* is believed to be important for *P. gingivalis* colonization of periodontal pockets. *P. gingivalis* long fimbriae (FimA) bind to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) on the streptococcal surface [31,32], while its short fimbriae (Mfa) specifically recognize SspB proteins on the *S. gordonii* cell surface and are required for the development of *P. gingivalis* biofilms on streptococcal substrates [33]. Mfa fimbriae are also important for autoaggregation and microcolony development, following the attachment of *P. gingivalis* to substratum via its major fimbriae [34]. Interestingly, *P. gingivalis fimA* is repressed upon contact with *Streptococcus cristatus* arginine deiminase (ArcA), inhibiting *P. gingivalis* surface attachment and polymicrobial biofilm formation [35,36]. This phenomenon has also been suggested as an intergeneric bacterial contact-dependent communication that negatively regulates *P. gingivalis* biofilm formation. The negative correlation of their distribution in the subgingival region also suggests that *S. cristatus* is an antagonistic partner against *P. gingivalis*, while its ArcA could be a potential candidate against *P. gingivalis* biofilm formation [37]. *P. gingivalis* outer membrane vesicles (OMVs) have been found to aggregate with the antecedent colonizers *Streptococci* spp., *Actinomyces naeslundii*, *Actinomyces viscosus* and *Fusobacterium nucleatum* [38]. *P. gingivalis* OMVs also mediate adherence of *P. gingivalis* to *T. forsythia* and aid *T. forsythia* in the attachment to and invasion of epithelial cells [39]. The ability of OMVs released by *P. gingivalis* to bind to and sequester antibacterial agents such as chlorhexidine, offers additional resistance and protection to other oral bacteria [40]. *P. gingivalis* HmuR serves as a major hemin uptake protein and adhesin for three-species community development with *S. gordonii* and *F. nucleatum*, but was unimportant for mono- or dual-species biofilm formation [41]. Hbp35 is another multifunctional hemin binding protein that contributes to hemagglutination,