Persistence of *Tannerella forsythia* and *Fusobacterium nucleatum* in dental plaque: a strategic alliance

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Abstract

Purpose of Review: The Gram-negative oral pathogen *Tannerella forsythia* is implicated in the pathogenesis of periodontitis, an inflammatory disease characterized by progressive destruction of the tooth supporting structures affecting over 700 million people worldwide. This review highlights the basis of why and how *T. forsythia* interacts with *Fusobacterium nucleatum,* a bacterium considered to be a bridge between the early and late colonizing bacteria of the dental plaque.

Recent Findings: The recent findings indicate that these two organisms have a strong mutualistic relationship that involves foraging by *T. forsythia* on *F. nucleatum* peptidoglycan and utilization of glucose, released by the hydrolytic activity of *T. forsythia* glucanase, as a nutrient by *F. nucleatum*. In addition, *T. forsythia* has the unique ability to generate a toxic and inflammogenic compound, methylglyoxal, from glucose. This compound can induce inflammation, leading to the degradation of periodontal tissues and release of host components as nutrients for bacteria to further exacerbate the disease.

Summary: In summary, this article will present our current understanding of mechanisms underpinning *T. forsythia-F. nucleatum* mutualism, and how this mutualism might impact periodontal disease progression.

Keywords

periodontitis; peptidoglycan; methylglyoxal; periodontal pathogens; glucans

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INTRODUCTION

My interest in *Tannerella forsythia* and oral microbiology in general began when I joined Dr. Robert Genco's laboratory as a postdoctoral fellow in 1991. His laboratory at the time was studying the molecular mechanisms of the periodontal pathogen, *Porphyromonas gingivalis*. One of the research projects was aimed at defining the role of a subunit protein, FimA, that forms hair-like structures known as fimbriae on the surface of *P. gingivalis,* in bacterial colonization as well as in inflammation. As my first project in the lab, I generated the full length recombinant FimA protein and its several truncated derivatives, and subsequently with the help of colleagues in the lab delineated the functional domains of the FimA protein responsible for binding to salivary proteins [1–3]. I will never forget the 'childlike' excitement and enthusiasm Dr. Genco showed when he first saw the Coomassie stained protein gel with the purified recombinant FimA protein band at an expected size! I experienced the same enthusiasm, curiosity and love for science that he had that day until his last breath! His passion for oral microbiology-immunology was infectious and I feel fortunate to have had the opportunity to learn from him in the subsequent years. Dr. Genco was a pioneering figure who influenced many individuals through his work ethic and dedication. I continued my interest in *P. gingivalis* fimbriae and co-authored many papers on the subject with Dr. Genco, such as on the potential of FimA peptides as vaccine antigens against *P. gingivalis*. The studies led to the development of live vaccine vectors based on the commensal oral streptococci *Streptococcus gordonii* for the delivery of FimA peptide antigens [4, 5]. As a proof of principle concept for a periodontal vaccine, we showed that via oral immunization with such genetically modified vectors expressing FimA protein it was possible to significantly block the *P. gingivalis*-associated alveolar bone loss in a rodent model [6].

A bacterium that caught my attention while working on *P. gingivalis* was *Bacteroides forsythus* - a 'new kid on the block' at the time - now known as *Tannerella forsythia*. This bacterium was isolated by Anne Tanner at the Forsyth Institute in 1979 as a slow growing fusiform-shaped bacterium from plaque samples of periodontitis patients [7]. The organism could grow on blood agar plates when *Fusobacterium nucleatum* was co-streaked alongside as a helper bacterium [8]. Subsequently, Wyss (1989) [9] showed that supplementation of the culture medium with *N*- acetylmuramic acid (MurNAc), an amino sugar that together with *N*-acetylglucosamine (GlcNAc) forms the repeating disaccharide backbone of the peptidoglycan layer, it was possible to support the growth of the organism. In 2001, based on 16S rRNA gene sequencing, the organism was placed in a new genus, *Tannerella*, and *T. forsythia* became the founding member of the genus [10]. The studies have demonstrated that *T. forsythia* existed in the oral cavity of humans at least since the prehistoric Neolithic times, and the organism has thus adapted well to its oral niche in modern humans [11]; it is also found in the oral cavity of cats [12] and dogs [13]. It remains to be determined what adaptive advantages the loss of ability to de novo synthesize peptidoglycan amino sugars MurNAc provide to the bacterium, except for possibly reducing the genomic burden to some extent. In the genus Tannerella, species that have also generated interest in recent years include the taxa designated as Tannerella sp. BU063 (also known as human oral taxon 286 [HOT 286]) [14] and *Tannerella* sp. BU045 (HOT 808) [15]. Though these spp. are found

frequently in periodontal disease-associated plaques, they have not been found to proliferate to high densities, and thus their role in the pathogenesis of periodontal diseases is debatable [16]. However, BU045 is more frequent in periodontitis, whereas BU063 is considered a 'periodontal health associated' species [17]. BU063 has recently been cultivable in the presence of helper bacteria [17] while BU045 remains to be cultivated.

T. forsythia is a major periodontal pathogen involved in the development of periodontitis. This is corroborated by its high abundance in periodontal disease pockets, its prevalence in patients with chronic periodontitis [18–21] and the fact that the organism is able to induce experimental periodontitis in rodent models [22, 23]. *T. forsythia, P. gingivalis* and *Treponema denticola* - a triad known as 'red- complex' - are organisms which are strongly associated in periodontitis [20]. However, as we realize now based on 16S rDNA bacterial identification schemes, there are potentially many more species associated with the disease [24] and the disease etiology involves a synergistic and dysbiotic complex community where red-complex bacteria likely play a significant role [25].

In this review, we present recent findings of our group highlighting the survival strategies of *T. forsythia* in the oral cavity, depending largely on interspecies metabolic exchange between *T. forsythia* and its dental plaque partner *F. nucleatum* and how this metabolic exchange might affect microbial ecology and periodontal immunity at large.

T. forsythia – F. nucleatum synergistic partnership.

A large body of clinical evidence implicates *T. forsythia* in the pathogenesis of periodontitis, a disease that leads to inflammatory alveolar bone loss and is a well-established risk factor of atherosclerosis, diabetes, and low weight or preterm births [26, 27]. Targeted gene deletion strategy [28] has facilitated the identification of many virulence factors of *T. forsythia* (reviewed in references [26, 29]); e. g., leucine-rich repeat BspA protein [30], sialidases [31], surface (S)-layer glycoproteins [32], matrix metalloprotease-like enzyme karilysin [33] and KLIK proteases [34], and dipeptidyl aminopeptidase IV [35]. Recent studies have also implicated *T. forsythia* in the etiology of endodontic and dental implant infections [36, 37]. Higher levels of *T. forsythia* have been reported in the dental plaque samples of post-menopausal obese women [38, 39] and the bacterium has also been isolated from women with vaginosis [40]. A recent case control prospective study implicated *T. forsythia* in the development of esophageal cancers [41]. In experimental settings using rodent models, *T. forsythia* has been shown to enhance periodontal bone loss when given as a polymicrobial infection with the red-complex species *P. gingivalis* and *T. denticola* [23]. *T. forsythia* has been also shown to induce foam cell formation and accelerate the formation of atherosclerotic lesions in mice [42].

F. nucleatum is an opportunistic pathogen and is considered a 'bridge bacterium' since it facilitates the development of dental plaque due to its ability to co-aggregate with both the early-and late-colonizing bacterial species [43, 44]. Moreover, in recent years *F. nucleatum* has gained much notoriety and attention due to its probable role in colorectal cancer development [45, 46]. *T. forsythia* has been shown to co-aggregate and form synergistic cobiofilms with *F. nucleatum* in vitro [47], and in human dental plaque the two species coexist in close physical proximity [48]. Moreover, co-inoculation of these two organisms

induces a synergistic increase in abscess formation in rabbits [49] and alveolar bone loss [50] in mice, as compared to infection with either organism alone. Thus, both in vitro and in vivo data suggest a mutualistic partnership between these two species.

Interspecies sensing and metabolite exchange.

Peptidoglycan scavenging.—As mentioned above, *T. forsythia* is auxotrophic for the peptidoglycan amino sugar, N-acetylmuramic acid (MurNAc). MurNAc is a unique amino sugar found only in the chemical make-up of bacteria. The peptidoglycan layer (cell-wall) is a polymer of alternating β−1,4 linked MurNAc and GlcNAc residues in which MurNAc is attached to L- and D-amino acid-containing stem pentapeptides that crosslink adjacent sugar chains [51]. The peptidoglycan layer is essential in all bacteria as it provides a rigid covering (exoskeleton) surrounding the cytoplasmic membrane, protects bacteria from osmotic shock, and determines the shape [52]. The MurNAc auxotrophy of *T. forsythia* is due to the lack of UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) and UDP-N-acetylenolpyruvoylglucosamine reductase (MurB) - key enzymes involved in the de novo synthesis of MurNAc from simple sugars in bacteria [53]. In order to cope with this critical deficiency, the bacterium relies on exogenous sources for obtaining the precursors (MurNAc or muropeptides) of peptidoglycan. For this purpose, *T. forsythia* has evolved committed pathways for transporting and recycling MurNAc and peptidoglycan fragments from its environment - the subgingival niche, where these components are abundantly released by cohabiting bacteria as a consequence of their cell-wall breakdown/restructuring during division and growth. We have recently shown that *T. forsythia* has evolved a novel transport system, MurTKQ, comprising an integral membrane protein (TfMurT), a sugar kinase TfMurK), and an etherase (TfMurQ) for the transport and utilization of exogenous MurNAc [54]. However, an important fact is that the human host cannot synthesize MurNAc and the cohabiting bacteria are the sole source of free MurNAc or MurNAc containing peptidoglycan fragments (muropeptides). Conceivably, peptidoglycan fragments released by cohabiting bacteria during their cell-wall turnover or death could be readily accessible to *T. forsythia* in the microbial communities. We recently showed in an in vitro model that *T. forsythia* can utilize muropeptides derived from *F. nucleatum* via transport through a muropeptide permease (TfAmpG) whose expression is regulated by a unique hybrid two-component system (Tf GppX) [55]. A close physical association of these two organisms in the dental plaque biofilms [48] and the fact that these organisms show synergistic co-biofilm activity in vitro [56] and virulence in a mouse model of periodontitis [50] suggest *T. forsythia's* dependence on *F. nucleatum* for peptidoglycan.

Glucan hydrolysis and methylglyoxal production.

We have recently shown that *T. forsythia* secretes a β-glucanase enzyme (TfGlcA) that can digest β−1,6 and β−1,3 linked β-glucans into glucose monomers and polymers [57]. Interestingly, the expression of this enzyme is induced when the bacterium comes in contact with *F. nucleatum*. The β-glucanase coding gene is part of an operon located immediately downstream of an operon that codes for a putative extracytoplasmic function (ECF) sigma factor and an anti-sigma factor regulatory proteins, which we predict are responsible for regulating β-glucanase expression in response to *F. nucleatum* and possibly other environmental stimuli [57]. Furthermore, glucose released from β-glucan hydrolysis

by β-glucanase serves as a source of carbon for *F. nucleatum* and promotes its biomass in mixed species biofilms [57]. However, this increased glucose availability does not promote *T. forsythia* biomass since the bacterium is asaccharolytic. Rather, glucose can become toxic to the bacterium due to its conversion into methylglyoxal (MGO) via a methylglyoxal synthase (MgsA) catalyzed pathway in *T. forsythia* [58, 59]. MGO is an electrophilic di-carbonyl compound which is toxic to both bacterial and host cells. In addition, MGO can covalently modify host proteins to generate glycation adducts known as advanced glycation endproducts (AGEs) which, via activation of RAGEs (receptors for AGEs), can induce inflammatory responses [60, 61]. Potentially, therefore, dietary intake of β-glucans (rich in oats and breads) might promote dental plaque development and periodontal inflammation. Interestingly, fusobacteria can benefit from their assocaition with *T. forsythia* in other ways as well. For instance, fusobacteria can metabolize silaic acid released by the action of *T. forsythia* NanH siliadase enzyme on host silaoglycoproteins (salivary mucins and cell surface proteins) as a carbon source and/or repurpose the sugar to cloak their surface with the sugar [31].

Potential consequence of peptidoglycan scavenging and MGO production on periodontal microbiota and inflammation.

While peptidoglycan plays structural and physiological roles in bacteria, its components are immunomodulatory in nature and can orchestrate host immunity [62–65]. Specifically, peptidoglycan fragments of both Gram-negative and Gram-positive bacteria can induce inflammation by activating the NOD (nucleotide binding and oligomerization domain) receptors expressed in innate immune cells such as monocytes, macrophages, neutrophils and epithelial cells. NODs, including NOD1 and NOD2, are a family of intracellular pattern recognition receptors that recognize peptidoglycan components [66–68]. NOD2 related signaling has been strongly correlated with inflammatory diseases such as Crohn's disease and the Blau syndrome [69–71]. The specific ligands for NOD1 and NOD2 receptors are the dipeptides D-γ-glutamyl-meso-DAP (iE-DAP) and MurNAc-L-Ala-D-isoGln (mMDP), respectively, which are released in the environment when bacteria breakdown and recycle their cell-wall during division [72, 73]. We hypothesis that *T. forsythia* associated muropeptide scavenging might lead to dampening of NOD2 signaling in the subgingival niche and *T. forsythia* in this scenario might act as a key modulator of local inflammation during periodontitis.

As mentioned above, another outcome of *T. forsythia-F. nucleatum* interaction is MGO production. A plausible role of *T. forsythia*-produced MGO in periodontitis is supported by clinical studies that show higher levels of MGO correlating with *T. forsythia* loads are present in gingival exudates from severely inflamed lesions of periodontitis patients [74]. Moreover, in vitro data suggest that free MGO can directly induce gingival connective tissue damage by causing apoptosis of gingival fibroblasts [75] and *T. forsythia*-secreted MGO can cause collagen glycation which in a RAGE-dependent manner can induce pro-inflammatory cytokine production [59]. In addition, subgingival bacteria that lack the abilty to detoxify MGO are likely to be susceptible to MGO. Taken together, MGO might be an important contributing factor in the development and severity of periodontitis by promoting both inflammation and microbial dysbiosis.

CONCLUSIONS

T. forsythia displays a unique affinity for *F. nucleatum* and the evidence suggests that this association is mutualistic and might be critical for the development of dental plaque, and hence in the progression of periodontal disease (summarized in a model in Figure 1). This mutualism is due to the following reasons. *T. forsythia,* is auxotrophic for the aminosugar N-acetylmuramic acid (MurNAc) which, along with N-acetylglucosamine (GlcNAc), forms the repeating disaccharide unit of the peptidoglycan backbone in most bacteria. To circumvent this deficiency, the bacterium must harvest peptidoglycan precursors from the environment for its cell wall biosynthesis and survival. The bacteriium has evolved well-committed pathways for the transport and recycling of MurNAc and peptidoglycan fragments released by the cohabiting bacteria during their cell wall turnover. In this regard, we believe *F. nucleatum* is a crucial partner of *T. forsythia* in the dental plaque. This relationship appears to be mutualistic and strategic as *F. nucleatum* can provide peptidoglycan fragments to *T. forythia* in return for glucose as a nutrient released by *T. forythia* β-glucanase action on glucans. Moreover, *T. forsythia* is a unique pathogen with an ability to produce methylglyoxal (MGO), an electrophilic di-carbonyl compound that can covalently modify host proteins to generate inflammogenic adducts known as advanced glycation endproducts (AGEs). MGO is also toxic to bacteria that lack detoxification systems, and thus MGO may aid in the release of peptidoglycan from such organisms. We hypothesis that MGO, by promoting the formation of AGEs and its killing activity against susceptible organisms, is involved in the microbial dysbiosis process. It would be interesting to determine how *T. forsythia*'s peptidoglycan scavenging activities and MGO production orchestrate a state of innate immune dysregulation and microbial dysbiosis in the oral cavity.

ACKNOWLEDGEMENTS

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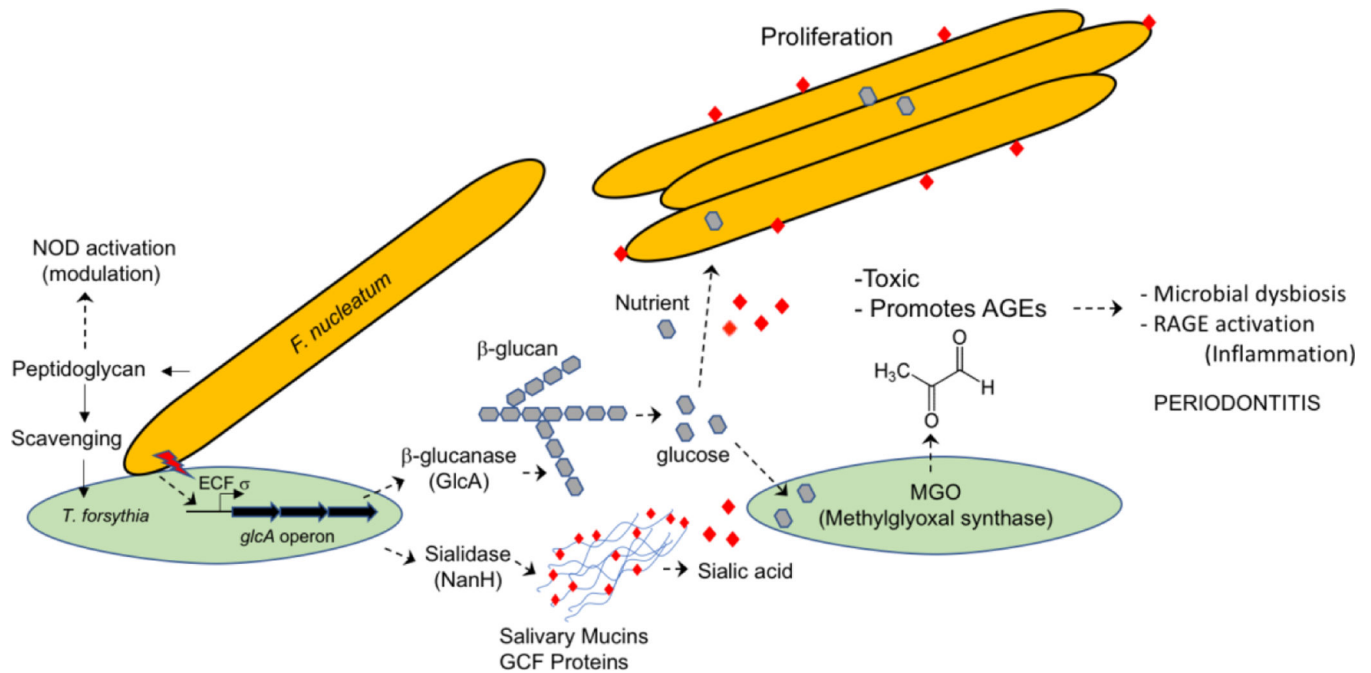
Figure 1. Model of *T. forsythia* - *F. nucleatum* interbacterial interactions in dental biofilm and their impact on periodontal ecology and inflammation.

T. forsythia utilzes peptidoglycan fragments released by *F. nucleatum* and possibly other bacteria for its survival. This is expected to reduce the levels of free peptidoglycan in the subgingival niche and dampen NOD (nucleotide bnding oligomerization domain containing intracellular pattern recognition proteins) - mediated inflammatory responses of the host. *F. nucleatum* triggers the secretion of β-glucanase from *T. forsythia* via an ECF σ-factor (extracytoplasmic function sigma factor) dependent induction of the *glcA* operon in *T. forsythia*. β-glucanase thus secreted can digest dietary β-glucans into glucose residues, which serve as a source of carbon for *F. nucleatum* and a precursor for the generation of methylglyoxal (MGO) by *T. forsythia*. MGO covalently modifies host proteins to generate AGEs (glycation adducts known as advanced glycation endproducts), which can trigger inflammation via the activation of RAGEs (receptor for AGEs). Moreover, sialic acid released from salivary mucins by *T. forsythia*-secreted sialidase enzyme NanH might be utilized by *F. nucleatum* to decorate its cell surface with the sugar. Figure keys: red lightning bolt. *F. nucleatum* stimulating ECF σ-factor in *T. forsythia*; red diamonds, sialic acid; grey hexagons, glucose.