12 Periodontal Pathogen Sialometabolic Activity in Periodontitis

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Periodontitis (PD) is an inflammatory disease characterized by progressive destruction of the tooth-supporting structures, often leading to tooth loss. It affects over 700 million people worldwide and is one of the major oral conditions globally after caries that is estimated to cost $442 billion per year [1–3]. PD is induced by a subgingival polymicrobial community in which a bacterial triad known as the red complex comprising of *Porphyromonas gingivalis* (Pg), *Treponema denticola* (Td), and *Tannerella forsythia* (Tf) is strongly represented. The environmental niche that these bacteria inhabit, as with most human mucosal infections and colonizable surfaces such as the airways, gut, and female reproductive tract, is rich in glycoproteins decorated with *N*- or *O*-linked sugar-glycan chains, of which most are capped at the terminal end with the 9-carbon sugar, sialic acid (Neu5Ac) [4]. A wide variety of pathogens from different genera inhabiting a range of niches within the body, including the oral cavity, are known to utilize Neu5Ac as a source of carbon and nitrogen or to cloak their surface to avoid immune attack (please see review [5]). Likewise, the red-complex bacteria have been shown to produce sialidase that cleaves the terminal sialic acid from glycoprotein-linked glycans on the surface of epithelial cells, immune cells, and oral secretions such as gingival crevicular fluid (GCF). In this review, we summarize accumulating evidence demonstrating that sialic acid harvesting by oral pathogens is not only key to their survival in the oral cavity but might also be responsible for immune dysfunction and disrup-

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tion of tissue integrity observed in periodontitis. Further, we propose that sialidase inhibition with sialidase-targeting pharmacological drugs, such as those currently employed for influenza and also from a range of other sources, may be an attractive adjunct therapy in controlling periodontitis. While sialidase production is a prominent feature of the red-complex bacteria, it is not limited to these species in the oral cavity. For example, several oral commensal and opportunistic organisms such as *Streptococci* spp. (mitis, oralis, intermedius), *Capnocytophaga* spp., *Actinomyces naeslundii*, *Actinomyces oris*, *Prevotella oralis*, *Bifidobacterium dentium*, and *Propionibacterium acnes* produce related sialidases [6, 7]. It is likely that both the nature of physical niche in the mouth and the physiology of individual species dictate the importance of sialidase activity in a given setting. Among the species that might be considered as early or primary colonizers of tooth surfaces in the mouth such as *Streptococci*, *Actinomyces*, *Bifidobacterium*, and *Propionibacterium* are saccharolytic organisms where their primary carbon source is often glucose or other dietary sugars, meaning sialidase activity might chiefly be present to access underlying sugars as part of a more versatile catabolic profile. However, as these organisms are not in direct contact with mucosal surfaces, or immune cells, the impact of their sialidases on inflammation is expected to be limited at best.

Moreover, some bacteria in the oral microbiota that do not produce their own sialidase likely benefit from sialidase activity of other bacteria. For example, some *Fusobacterium* spp. can catabolize exogenous sialic acid for energy or reprocess sialic acid to decorate their surface with the sugar [5]. Still others can also benefit from community sialidase activity, which might allow them to utilize sialic acid or bind to underlying sugars (cryptic epitopes) on glycans, e.g., *S. gordonii*, *S. mutans*, *S. sanguinis*, and *S. salivarius* [7–10]. The contribution of sialidase activity in periodontal inflammation has recently come to light from clinical observations of raised levels of sialidase activity in GCF of periodontitis patients [11]. The negative impact of sialidase activity on periodontal disease is heightened since in the context of the subgingival environment of the periodontal pocket, sialidase activity can disrupt the integrity (structure-function) of host glycoproteins, disrupt pattern-recognition receptor (TLRs) signaling in infiltrating immune cells as well as epithelial layers, and promote the survival, persistence, and pathogenesis of periodontal bacteria.

12.1 Sialic Acid Foraging by Periodontal Pathogens

Current evidence indicates that the key periodontal pathogens *P. gingivalis*, *T. denticola*, and *T. forsythia* strictly rely on host-derived sialic acid for their survival in the oral cavity and virulence. This is unlike many other human pathogens such as *Campylobacter jejuni*, *Neisseria meningitidis*, and some *Fusobacterium* spp. that have dedicated biosynthetic pathways to synthesize their own sialic acid [5]. The work from our groups showed that *T. forsythia* mutants lacking sialidase and the sialic acid transporter NanT are unable to acquire environmental sialic acid and are severely attenuated in biological activities such as survivability in biofilms on sialoglycoprotein substrates [12] and interactions with and survival on epithelial cells [13, 14]. In addition, other groups have shown that sialidase deletion in a capsulated strain of *P. gingivalis* results in reduced capsule thickness phenotype [15] and gingipain protease expression [16]. These studies showed that compared to the wild-type capsulated strain, a sialidase-deficient mutant in vitro formed less biofilms and was less resistant to killing by the host complement [15]. Moreover, while the wild-type strain was able to spread to multiple organs and cause mouse mortality following subcutaneous infection, the sialidase-deficient mutant was found to be highly attenuated showing only localized spreading and ineffective in causing mortality in mice. In the case of *T. denticola*, sialidase deficiency caused an increased surface deposition of complement attack complex and reduced virulence in a mouse model [17]. In addition, though it is yet to be established in the periodontal setting, our groups have preliminary evidence of sialidase-dependent modulation of

epithelial and monocyte responses to LPS stimulation, and as such sialic acid scavenging might exacerbate inflammation by affecting TLR signaling. In this scenario, periodontal pathogen-secreted sialidases might contribute to subgingival inflammation through increasing TLR4 responsiveness to LPS as it has been shown that desialylation of TLR4 promotes its dimerization and activation at other human mucosal layers [18]. Moreover, sialidase-mediated desialylation is a mechanism through which one of the immunosuppressive circuits is compromised in immune cells dependent on Siglecs (sialic acid-binding immunoglobulin-type lectins) [19]. In a normal homeostatic state, Siglec-G/10 (G, mouse/10, human) binds a sialylated receptor CD24 and induces an inhibitory circuit that attenuates TLR signaling. Indeed, the data from our lab show that *T. forsythia* sialidase treatment in vitro can increase TLR4 activation in macrophages (unpublished). Thus, sialic acid utilization by pathogens can take roles in immune evasion and dysregulation, community biofilm development, and pathogen survival. Plausibly, the sialidase activity might also assist periodontal pathogens in mitigating the toxic effects of ROS (reactive oxygen species) in the inflamed periodontal pockets as released sialic acid residues can act as scavengers of peroxide residues [20, 21]. Taken together, these findings demonstrate that sialidase is an important and common virulence determinant that may contribute to the pathogenicity of periodontal pathogens.

As mentioned above, both *P. gingivalis* and *T. denticola* possess sialidases that have been shown to be key to their survival and virulence [15–17]. Both *P. gingivalis* and *T. denticola* sialidases display a similar domain organization with a C-terminal catalytic domain with homology to glycosyl hydrolase 33 (GH33) family carbohydrate-active enzymes in the CAZy database [22] that is preceded by an N-terminal domain, which in the case of *T. denticola* is a putative peptidoglycan binding domain [17] while in the case of *P. gingivalis* shows no homology to any of the known protein domains in the sequence or structural databases (Fig. 12.1). Despite clear data existing that *T. denticola* seems to require sialidase activity or monomeric sialic acid for growth in serum, its genome sequence lacks any homologs of sialic acid catabolic genes [17], suggesting the existence of novel pathways.

In contrast, biochemical and functional knowledge of the *T. forsythia* NanH sialidase is well advanced, mostly from work in our teams. As with *P. gingivalis* and *T. denticola*, we have revealed that *T. forsythia* sialidase is key to the bacterium's microbiology and virulence [5, 12–14]. However, unlike *P. gingivalis* and *T. denticola*, it expresses its sialidase, NanH, encoded by the *nanH* gene, as part of a dedicated sialic acid scavenging, transport, and utilization operon (Fig. 12.1). Of note, this operon is present in all of the *T. forsythia* strains sequenced to date, including the genomes of three clinical isolates

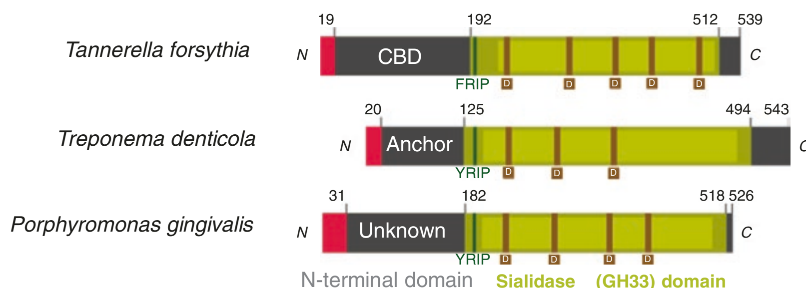

Fig. 12.1 Schematic representation of periodontal pathogen CAzymes. A. sialidases: The GH33 sialidase domains are shown in yellow, with the Asp (D) boxes indicated alongside the catalytic FRIP domains. Signal sequences are indicated in red. N-terminal domains are indicated in gray, with the *T. forsythia* confirmed carbohydrate-binding domain (CBD); *T. denticola* putative cell wall anchor (Pfam PF09479) indicated; alongside the *P. gingivalis* N-terminus, which has no homology in databases

recently sequenced by our group [23] and in metagenomic reconstruction from ancient calculus [24]. Notably the *nan* operon is missing from the related *Tannerella* BU063 strains isolated to date. NanH is a 62 kDa secreted enzyme again comprising two domains with the C-terminal domain comprising a catalytic domain that is a member of the GH33 family mentioned above. It contains five Asp-box motifs, a conserved catalytic Arginine triad, and FRIP-motif (Fig. 12.1). Once again *T. forsythia* NanH possesses a 170–180-amino-acid-long, N-terminal domain with no sequence or structural homology in the PDB database outside of sialidase enzymes found within members of the *Bacteroidetes*. The NanH N-terminal domain has now been characterized by our groups as a novel carbohydrate-binding module (CBM) with broad specificity for host glycans but which prefers sialylated glycans and those with alpha-2,3 glycosidic linkages [25]. As mentioned above, NanH is associated with a novel sialic acid utilization system which contains a novel transporter system (NanOUT) [26–28] for the uptake of monomeric sialic acid and a 9-0-sialate-acetylesterase (NanS) [26]. This NanS enzyme is novel in its class as it contains two SGNH-like Sialate-esterase domains and acts to enhance release of Neu5Ac (sialic acid) from host glycoproteins containing diacetylated sialic acids (Neu5,9Ac) that block the action of sialidases (ref). Furthermore, in the *nan* operon, NanH is followed by a β-hexosaminidase [29], which may act to release subsequent sugars within host glycans [26], and a predicted sialic acid mutarotase that improves utilization of the alpha anomeric form of sialic acid [30].

In addition, our studies have shown that the release, transport, and utilization of sialic acid are critical to the interaction and survival of *T. forsythia* with epithelial cells as well as bacterium's growth in glycoprotein-based biofilms [12–14, 29]. These data shed light on the ability of the bacterium to cleave and utilize host Neu5Ac as a survival strategy whereby removal of terminal sialic acid residues in host glycoproteins in salivary secretions and on epithelial cells allows the bacterium to colonize and utilize the liberated sialic acid as a source of carbon and nitrogen and possibly as a precursor for the peptidoglycan (PGN) synthesis (Fig. 12.2). This is pertinent as PGN synthesis pathways are notably lacking in *T. forsythia*, which cannot de novo synthesize the PGN amino sugar *N*-acetylmuramic acid (MurNAc) from simple nonamino sugars. While

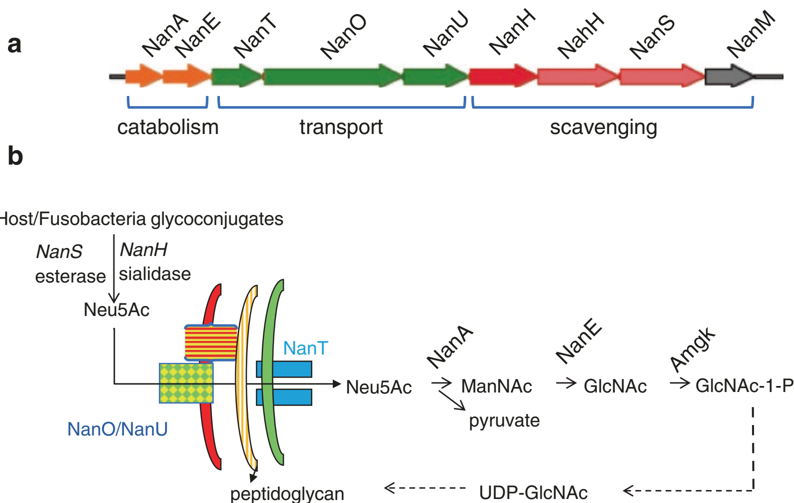

Fig. 12.2 Sialic acid scavenging as a means of peptidoglycan biosynthesis and survival by *T. forsythia*. (**a**) Genetic organization of sialic acid utilization operon. (**b**) Putative metabolic pathway of sialic acid utilization to peptidoglycan biosynthesis. Dashed line arrows indicate canonical enzymes involved in the conversion of various sugar intermediates to peptidoglycan have not been found in *T. forsythia*

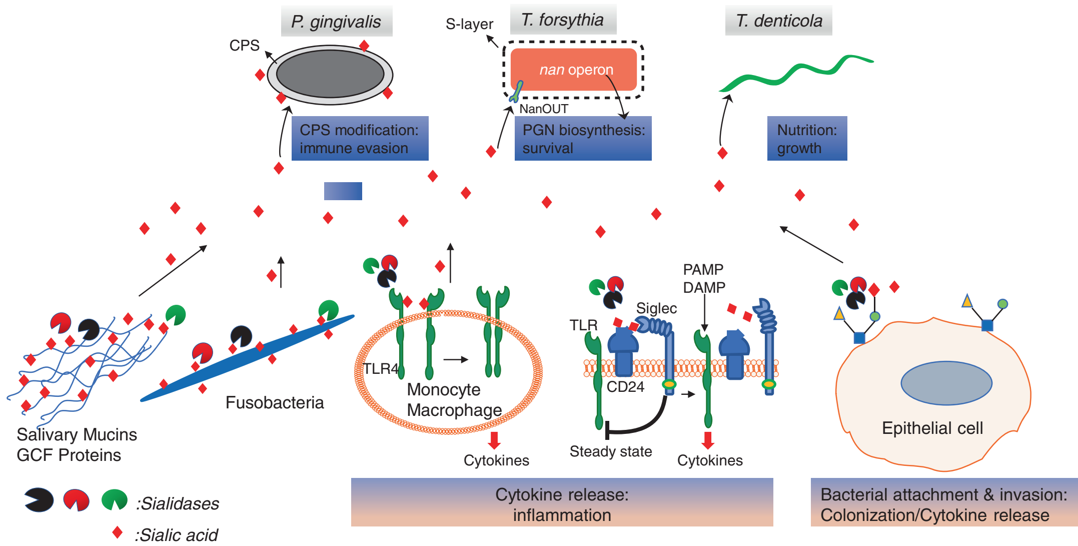

Fig. 12.3 Model of sialic acid-dependent lifestyle of periodontal pathogens. Key: *CPS* capsular polysaccharide, *PGN* peptidoglycan, *S-layer* surface layer, *NanOUT* sialic acid transporter system

it is able to uptake muropeptides released by other bacteria in the oral cavity, e.g., *Fusobacterium nucleatum* [31], we postulate that the role of *T. forsythia* sialidase in liberating free monomeric sialic acid might be critical for the bacterium's survival in the oral cavity as sialic acid could serve as an alternative source for MurNAc synthesis as we have suggested previously [28]. This is significant as the human host is also unable to synthesize and thus provide MurNAc to the bacterium. We postulate that by harvesting sialic acid as an alternative nutritional source, *T. forsythia* is able to gain a competitive edge over the subgingival cohabiting microbiota. In support of a notion that sialic acid foraging by *T. forsythia* might be critical in this respect, a community-wide transcriptome analysis of the subgingival microbiome has indicated that the *T. forsythia nanH* transcript levels likely increase in subjects with periodontitis as compared to healthy controls [32].

One by-product of this use of sialic acid would be a potential increase in availability of peptidoglycan fragments comprising NOD (nucleotide-binding oligomerization domain)-like receptor ligands such as muramyl dipeptide (MDP; NOD2 ligand) and γ-D-glutamyl-meso-diaminopimelic acid (iE-DAP; NOD1 ligand) [33] in the subgingival environment which may heighten NOD-mediated inflammation. Thus, sialic acid scavenging may potentially exacerbate inflammation by affecting TLR signaling as well as putatively increasing NOD-mediated inflammation.

Taken together, sialic acid cleavage by periodontal pathogens contributes to immune dysregulation and evasion, community biofilm development, and pathogen survival (Fig. 12.3). Importantly, support for the notion that sialic acid acquisition by the subgingival microbiome might be important in disease progression comes from our studies demonstrating a heightened expression of microbial sialidase activity in diseased sites compared to healthy sites and the heightened sialidase activity as a predictor of poor standard treatment outcomes [11].

12.2 Global Sialidase Activity and Impact on Health and Disease

It is important to take into account the contribution of sialidase activity of commensal oral organisms which may be critical for microbial ecology and health or disease in ways that have yet to be fully understood. In this regard, the sial-

idase activity of commensal bacteria may also be required for their growth/colonization and thus may have roles in maintaining healthy microbiota. The damaging effects of sialidase from commensal bacteria colonizing the supragingival niche might be mitigated both due to sialic acid-rich salivary mucins acting as a coating layer on hard surfaces. The delicate balance between the host and the sialidase activity in health is thus likely maintained by appropriate host barrier functions of salivary proteins including mucins and innate immune responses. This is in contrast to the subgingival niche and inflamed periodontal pockets where sialidases from periodontal pathobionts can directly encounter infiltrating immune cells (monocytes/macrophages) and influence inflammation. In addition, as mentioned above, sialidase activity might promote the survival of pathobionts in the harsh subgingival environment; in the case of *T. forsythia*, sialidase activity promotes its growth, and for *P. gingivalis* and *T. denticola*, sialidase activity is important in protecting these bacteria against the complement attack while it may also allow them easier access to underlying protein substrates that are key for their virulence and nutrition [15, 17]. Thus, the role of sialidase might be context dependent. In a periodontitis setting, inflammation and dysbiosis due to pathobionts *P. gingivalis*, *T. forsythia*, and *T. denticola* and proliferation of commensals might further increase global sialidase activity and thus synergistically exacerbate periodontitis. This notion is supported by observations that sialidase activity is elevated in plaque biofilms [34] and is high in diseased GCF in patients [11].

12.3 Translational Potential of Sialidase Inhibition

The dependence of key periodontal pathogens on sialic acid for survival, virulence, and immune modulation provides treatment opportunities for periodontitis by targeting sialidase activity with sialidase inhibitors. The potential translatability of blocking periodontal pathogen sialidase activity, heightened by the availability of several current FDA-approved (Tamiflu, Relenza, Peramivir) or novel sialidase inhibitors, to alleviate periodontitis and associated dysbiosis development and inflammation is very promising. Sialidase inhibitors could be used as an adjunct therapy in situations where periodontal pathogens present resistance to antibiotics. This may also minimize the use of antibiotics and development of antibiotic resistance. Our published and preliminary studies have demonstrated that inhibiting sialidase function with pharmacological inhibitors (FDA-approved zanamivir and oseltamivir) can block the ability of *T. forsythia* to form biofilms and survive on sialoglycoconjugates and epithelial cell monolayers, both in mono-species and mixed-species infections with *P. gingivalis* and *Fusobacterium* spp. [12, 14]. Importantly, sialidase inhibition can block the availability of sialic acid for peptidoglycan biosynthesis in *T. forsythia*, and therefore may reduce any selective advantage *T. forsythia* might have over other bacteria in biofilms in vivo. In the case of *P. gingivalis*, lack of sialidase activity in the bacterium results in increasing sensitivity to hydrogen peroxide and reduced gingipain protease activity, suggesting that sialidase activity might be involved in regulating the virulence potential of this keystone pathogen [16]. In a capsulated strain of *P. gingivalis*, sialidase activity seems to influence capsule formation and confer the bacterium the ability to resist complement attack [15]. Moreover, based on in vivo studies in a mouse model, the role of sialidase activity has been highlighted. It has been shown that while wild-type *P. gingivalis* has the ability to disseminate to multiple organs following infection, sialidase deficiency abrogates this ability causing only localized spreading around the site of infection [15]. In *T. denticola*, sialidase activity seems to be responsible for the acquisition of sialic acid as a nutrient as well as for sialic acid modification of surface glycoproteins as a means of blocking deposition of membrane attack complex and killing [17]. An obvious next step is therefore to test the in vivo potential of these synthetic as well as other naturally occurring plant-derived inhibitors (berberine and palmatine) [35] against sialidase-producing pathogens. In this regard, it is tempting to first test the efficacy of sialidase inhibitors/

inhibitor cocktails found effective in vitro in conferring protection to pathogen-induced periodontal bone loss in a mouse model, including in polymicrobial situations. The inhibitors might include both those that block the NanH sialidase's enzymatic activity (Tamiflu) and inhibitors that block NanH's lectin function (S-Lewis oligosaccharides or glycan mimics). The use of sialidase inhibitors for the treatment of periodontitis is not without risks due to potential off-target effects of these inhibitors on endogenous host sialidases (neuraminidases) playing important roles in the physiology of the host [36–38]. However, in principle one can design specific inhibitors that target the bacterial sialidases while not compromising functionality of the host sialidases [39]. We envisage a situation where topical administration of glycosidase inhibitory compounds could be deployed as gels applied as part of nonsurgical root debridement or within mouthwashes.

12.4 Summary

In this review we provided accumulating evidence demonstrating that sialic acid foraging by oral pathogens is not only key to their survival in the oral cavity but is also responsible for immune dysfunction and dysbiosis observed in periodontitis. We envisage that sialidase neutralization with pharmacological drugs, such as those currently employed for influenza, and also plant derived sialidase inhibitors in development may be an attractive adjunct therapy in controlling periodontitis.

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