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Treponema denticola interactions with host proteins

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Oral *Treponema* species, most notably *T. denticola*, are implicated in the destructive effects of human periodontal disease. Progress in the molecular analysis of interactions between *T. denticola* and host proteins is reviewed here, with particular emphasis on the characterization of surface-expressed and secreted proteins of *T. denticola* involved in interactions with host cells, extracellular matrix components, and components of the innate immune system.

Keywords: *spirochetes*; *virulence factors*; *periodontal disease*

Approximately 30% of the US adult population has at least one periodontal site with demonstrable gingival recession and bone loss (1). In contrast to healthy gingival plaque in which the complex microbial community dominated by facultative Gram-positives, periodontal lesions harbors a microflora dominated by proteolytic Gram-negative anaerobes and spirochetes (2, 3). While a consortium of three organisms denoted as the 'red complex' (*Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*) has the highest association with periodontal disease severity (4), spirochetes in particular are associated with severe and refractory periodontal conditions (2, 5–8). In periodontal lesions, spirochetes preferentially localize in the deepest part of the 'pocket,' specifically at the interface between the subgingival plaque and the epithelium (9). In advanced periodontitis, their ability to disrupt intercellular junctions likely contributes to invasion of underlying tissue (10–14). *T. denticola*, the most readily cultivable oral spirochete, is the model organism for studying both the unique biological features of these organisms and *Treponema*–host interactions in periodontal disease. Virulence-associated behaviors (15, 16) of *T. denticola* include adherence to host tissue and other microbes, motility, chemotaxis, tissue penetration, and release of immunomodulatory and cytopathic factors (reviewed in (17)). The numerical prevalence of spirochetes in disease, along with their high association with and location within diseased sites, strongly suggests that they play an important role in disease initiation and progression (4, 6, 9, 18).

Oral spirochetes, including *T. denticola* and over 50 other *Treponema* species, present unique challenges and opportunities to the field of molecular and cellular microbiology. Foremost among these challenges is the fact that at least 80% of oral *Treponema* spp. have never been cultured, and only *T. denticola* has been studied in detail. These nutritionally fastidious anaerobes possess features unique among bacteria, including cellular structure (19), motility apparatus (20), biosynthetic pathways (21), and outer membrane protein complexes (22, 23). As commensal residents and opportunistic pathogens of oral mucosal tissue, they offer a wide range of potential avenues for research into microbe–host interactions and signaling, microbial communities, microbial physiology, and molecular evolution. Thus, molecular level studies of oral spirochetes are timely and of high importance in understanding chronic bacterial infections such as periodontal disease.

Treponema denticola exists in a complex, multispecies biofilm environment in the gingival crevice. Numerous interbacterial interactions required for development and maintenance of the subgingival microbial community have been documented or proposed (24). These dynamic interactions comprise only part of the total of the environmental milieu in which these organisms have evolved. The oral microbiota live in a host mucosal environment consisting of several host cell types and extracellular matrix (ECM) components as potential substrates in addition to a fluid environment consisting of a complex and variable mix of saliva, gingival crevicular fluid, and serum components, including numerous antimicrobial components of both the innate and adaptive immune systems. As obligately host-associated organisms, oral spirochetes are extremely well adapted to

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survival in a eukaryotic host environment. This is reflected, as in many other host-associated microbes, in the relatively large number of *T. denticola* genes that can be clearly identified as having been acquired by horizontal gene transfer from an ancestral eukaryotic host (25–27). To understand the factors that allow commensal organisms to induce pathogenic responses under certain host environmental conditions, it is necessary to understand how they survive without causing disease. The focus of this review is on the interactions between *T. denticola* and host components that mediate both its persistence in the oral environment and its pathogenicity in periodontal disease. Primary attention will be given to interactions that are at least partially characterized and understood at the molecular level, and understudied areas will be pointed out where appropriate.

Research on oral spirochetes has progressed in recent years, driven partly by completion of the *T. denticola* genome sequence (25). Recent online release of the *T. vincentii* provisional annotated genome (http://www.ncbi.nlm.nih.gov/genomeprj/55865) and the unassembled genome sequence contigs of *T. lecithinolyticum* (28) have expanded the genomic resources for this group of oral microbes. Additionally, the Human Oral Microbiome Project is in the process of sequencing several other *T. denticola* strains (29). However, progress in molecular analysis of specific *T. denticola* behaviors has been considerably slowed by the limitations of currently available genetic systems for this organism, including extremely low transformation efficiency, few selectable markers (30), lack of reliable plasmid or other vectors for the most studied strain (31, 32), and lack of promoter-reporter systems. These significant technical issues, combined with the small number of researchers and the relatively low level of funding in this field, are continuing impediment to progress. This is reflected in the number of journal articles published on oral spirochetes relative to other periodontal pathogens. In 2010, approximately five times as many papers were published on *P. gingivalis* than were published on all oral spirochetes, including *T. denticola*, a ratio that has changed only slightly over the past 15 years.

Interactions with host cells

Exposure of cultured cells of various types to *T. denticola* (Fig. 1) results in: monolayer detachment and proliferation inhibition (33–35), plasma membrane fibronectin degradation (36), membrane blebbing, decreased intercellular contact and cytoskeletal rearrangements (12, 35, 37), and loss of volume control (37). Most studies prior to the advent of molecular cloning and genome sequencing did not identify the specific *T. denticola* components responsible for the observed cellular responses. One example of a study that made some limited progress in this regard is contained in a series of reports by Shenker and coworkers on the antiproliferative effects of *T. denticola* on fibroblasts and monocytes (33, 38, 39). In these studies, certain protein fractions of *T. denticola* were identified as containing the active agents, but the identities of the proteins have not yet been determined.

Studies over the past 15 years utilizing purified proteins and isogenic *T. denticola* mutants have begun the process of identifying specific molecular pathways responsible for these rather complex cellular responses and, in some cases, have identified host proteins interacting with *T. denticola* effector proteins (Table 1). Two of the most prominent and widely studied outer membrane components of *T. denticola* are the major surface protein

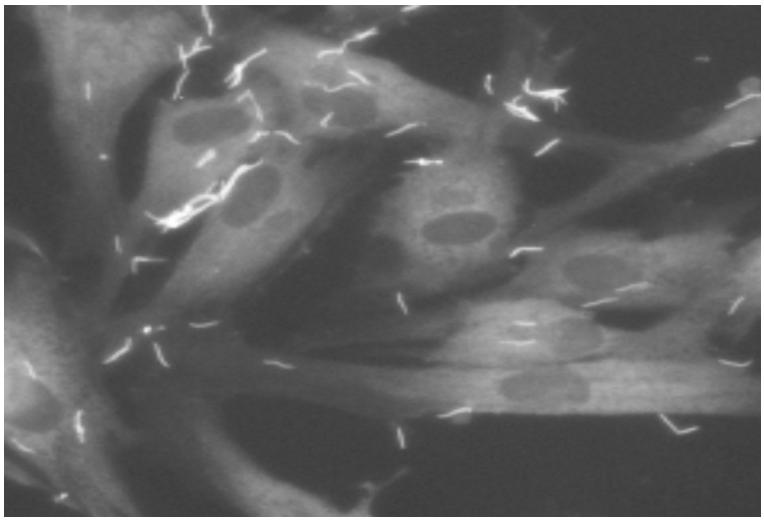

Fig. 1. Immunofluorescence micrograph showing *T. denticola* adherence to periodontal ligament cells. Periodontal ligament cell monolayers were challenged with *T. denticola* 35405 for 2 hours, then washed extensively with PBS before staining. Periodontal ligament cells are stained with phalloidin. *T. denticola* is visualized with Alexa-fluor-labeled antiMsp antibodies.

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Table 1. Molecular analysis of *T. denticola* interactions with host components, using purified proteins and isogenic mutants

Behavior	Protein(s) identified	Native activity[a]	Gene(s)	Recombinant activity[a]	Mutants	Mutant phenotype[b]
Motility	n/d	n/d	*fliFGH flgB, C, E, F*	n/d	*flgE tap1*	Non-motile (30, 140)
Chemotaxis	n/d	n/d	*cheAWXY dmcA, dmcB*	n/d	*dmcA, dmcB, cheA*	Non-motile, non-chemotactic (102, 141, 142)
Adherence to						
Eukaryotic cells	dentilisin	+ (40, 116)		n/d		
ECM components						
Fibronectin	Msp	+ (22)	*msp*	+ (67)	*msp* (143)	n/d
	OppA	+ (68)	*oppA-F*	n/d	*oppA*	↓ binding (68)
Fibrinogen	dentilisin	+ (100)		n/d	*prcA-prtP*	n/d
	Msp	+ (22)	*msp*	n/d	*Msp*	no change (143)
Plasminogen	OppA	+ (68)	*oppA-F*	n/d	*oppA*	↓ binding (68)
	Msp	n/d	*msp*	n/d	*msp*	↓ binding (68)
Laminin	Msp	+ (22)	*msp*	+ (67)	*Msp*	n/d
Hyaluronan	dentilisin (79)	n/d	*msp*	+ (66)		n/d
Keratin, collagen, heparin			*msp*	+ (66)		n/d
Cytotoxicity	Msp	+ (40)	*msp*	+ (40)	n/d	
	dentilisin	+ (40)	*prcB-prcA-prtP*	n/d		
Complement resistance (FH mediated)	FhbB	binds FH (115)	*fhbB*	+ (123)	*fhbB*	↓ FH binding; ↑serum sensitivity (124)
	dentilisin	cleaves C3 (122) cleaves FH (115)	*prtP*	n/d	*prtP*	↓ C3 cleavage (122); No FH cleavage (115)
Abscess formation	dentilisin	n/d	*prtP*	n/d	*prtP*	↓abscess formation (144)
	dentipain	n/d	*ideT*	cleaves β-chain insulin (133)	*ideT*	↓abscess formation (133)
Iron acquisition	Hemin binding proteins	n/d	*hbpA, hbpB*	n/d	*hbpA, hbpB*	↓ growth (145)

[a]Activity demonstrated [+, (citation)] or not reported [n/d].
[b]Reported mutant phenotype (citation) or not reported [n/d].

(Msp) and the PrtP protease complex (dentilisin, chymotrypsin-like protease (CTLP)) both of which have cytopathic effects in epithelial cells (40). Interestingly, although challenge with either Msp or dentilisin results in lysis of epithelial cells, Msp is hemolytic, whereas dentilisin is not (40), suggesting distinctly different mechanisms of interaction with different host receptor molecules.

Msp cytotoxicity is believed to be due to its demonstrated pore-forming activity in both planar lipid bilayers and in epithelial cell membranes (41, 42). This is consistent with the demonstrated lytic pore-forming activity of porins of other Gram-negative pathogens, including *Neisseria* spp. (43, 44). Interestingly, fibroblasts appear to be comparatively tolerant of Msp challenge, making them more tractable candidates for analysis of cellular responses. Studies by Ellen and coworkers have demonstrated that Msp disrupts the actin cytoskeleton of gingival fibroblasts, with specific effects, including disruption of actin stress fibers (45) and induction of *de novo* synthesis of subcortical actin filaments (35, 37, 46, 47). A specific peptide sequence within Msp has been shown to promote stress fiber formation (48, 49). Concurrently, and presumably related to this phenomenon, Msp blocks the ability of fibroblasts to regulate intracellular calcium gradients (50), which may contribute to the rather diverse range of morphological responses observed. The molecular mechanism of Msp-dependent calcium dysregulation, including identification of cellular Msp receptor(s), remains to be determined.

Dentilisin, a complex of three lipoproteins encoded by the *prcB-prcA-prtP* locus, has a wide range of cytopathic effects on whole cells and tissue. These effects are at least primarily due to its potent proteolytic activity of dentilisin, although as in other spirochetes, lipid moieties of the protease complex components may contribute to activation of innate immune responses (51). In particular, the ability of dentilisin activity to induce cell shrinkage and increased permeability of intercellular junctions have been shown to be a key factor in penetration and translocation of *T. denticola* through cell monolayers and ECM models (12, 13, 52). Further discussion of dentilisin's role in interaction with host proteins is found in subsequent sections dealing with interactions with specific host components.

T. denticola induces the production of various cytokines, including interleukin-1ß (IL-1ß), IL-6, IL-8, and TNF-α from various cell types (53–56). The specific *T. denticola* molecule(s) responsible have not yet been clearly identified. On the other hand, two of these studies (53, 55) also suggested that *T. denticola* hydrolyzes IL-8. Using wild-type and *prtP* mutant *T. denticola* strains, Miyamoto et al. showed that dentilisin activity was responsible for reduction of protein levels of inflammatory cytokines, including IL-1ß, IL-6, and TNF-α, detected in peripheral blood mononuclear cells following *T. denticola* challenge. This study highlights the difficulties encountered by investigators examining cellular responses to *T. denticola*. For this reason, it is important to include analyses of both protein expression and gene transcription when analyzing cellular responses that may be both induced by and subsequently modulated by proteolytic activity.

Interactions with ECM components

The ability to bind to or degrade ECM components is a hallmark of numerous mucosal pathogens. In addition to the direct tissue-destructive effects of ECM degradation, there is increasing evidence that degradation products of ECM components act as proinflammatory signals.

Fibronectin

Fibronectin is a dimeric, multidomain glycoprotein bound to the surface of eukaryotic cells and is a key component of the ECM. The molecular differences between the two major forms of FN (cellular FN and plasma FN) are due to differential mRNA splicing, and the two forms have distinct yet overlapping roles in formation and maintenance of ECM and in pathways of tissue homeostasis (reviewed in (57)). Numerous microbes, both commensal and pathogenic, exhibit FN-binding activity as a prominent behavior important for survival in the host environment. Molecular analysis of FN-binding proteins of several pathogens continues to be an important area of pathogenesis research over 30 years after the seminal discovery of FN binding to *Staphylococcus aureus* (58). Henderson et al. recently published an extensive review of FN function and the mechanisms and consequences of FN binding mediated by bacterial FN-binding proteins (59). Although the importance of *T. denticola* binding to FN has long been recognized, it has been studied rather less than the interaction between *T. pallidum* and FN. Due to the combination of the genetic similarities between *T. pallidum* and *T. denticola* and the complexities of working with *T. pallidum*, several investigators have studied *T. denticola* both in terms of periodontal pathogenicity and as a general model for *Treponema*–host interactions (60). Several key studies by the Ellen group beginning in the early 1990s established the role of FN in *T. denticola* interaction with host tissue. Dawson et al. characterized *T. denticola* binding to immobilized FN and proposed that one or more *T. denticola* FN-binding proteins were preferentially localized at the tips of spirochete cells (61, 62). In some strains, antiFN antibodies block *T. denticola* adherence to FN, further supporting the specificity of the binding interaction (63). Concurrent studies demonstrated the ability of *T. denticola* to induce degradation of cellular FN on gingival fibroblasts (36) and to induce cytoskeletal

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rearrangements that may be the result of FN-mediated signaling (37, 64, 65).

Although over 100 bacterial FN-binding proteins have been reported, the level of knowledge of these proteins and their interactions with FN vary widely. Detailed information on the structure of complexes formed between the bacterial proteins and FN is available in very few cases. In many instances, the only information available is that a protein binds FN in an *in vitro* assay, with or without information on kinetics, specificity, or FN target domain (extensively reviewed in (59)). It is rather common for bacteria to possess multiple distinct FN-binding proteins. Both experimental and genetic evidence suggests that *T. denticola* possesses at least two and many as nine or more proteins with FN-binding activity. Both Msp (22, 66, 67) and OppA (68) are reported to bind immobilized FN. Edwards et al. reported that Msp binds the N-terminal heparin I/fibrin I domain of FN (66) that is the most commonly reported target of bacterial FN-binding proteins. Although the specific domain targeted by OppA is not known, it is of interest that in ligand blotting experiments, both wild-type and Msp mutant *T. denticola* cells bind the same N-terminal FN domain (J.C. Fenno, unpublished observations).

Because *T. denticola* and *T. pallidum* survive in analogous mucosal environments and share many closely homologous proteins, it is reasonable to hypothesize that similar proteins might be involved in both microbes' demonstrated FN-binding activity. Two of 10 potential adhesins predicted by genomic analysis of *T. pallidum* showed FN-binding activity when expressed in *Escherichia coli* (69). One of these (TP0155) shows 45% identity with *T. denticola* TDE2138, whereas the other (Tp0483) is not homologous with any *T. denticola* protein. Like TP0155, TDE2318, six other predicted *T. denticola* proteins contain an M23 peptidase domain. Bamford et al. recently reported recombinant expression and FN-binding activity of five of these seven *T. denticola* predicted FN-binding proteins. Although immunofluorescence microscopy suggested surface localization of at least one of the *T. denticola* proteins, three of the deduced protein sequences lack predicted signal peptide sequences and may not be secreted (70). It is not yet known whether or under what conditions all of the genes encoding these predicted FN-binding proteins are expressed. These intriguing results support continued research into the mechanisms of *T. denticola*-FN interactions.

One issue in research into bacterial–FN interactions is the distinction between plasma and cellular FN. Many studies of FN binding use plasma-derived FN (largely due to its availability and price) when modeling behaviors in which cellular FN may be more relevant. In the simplest analysis, FN plays important roles in blood clotting and wound healing, whereas cellular FN is incorporated into the ECM as a part of a fibrillar matrix (59) and is a target for bacterial adherence (59). However, interaction of *T. denticola* with fibroblasts and related cell types results in fragmentation and degradation of cellular FN (36, 71), and the resulting fragments may have modulatory effects on tissue homeostasis and inflammation (72–74). *In vitro* studies showed that purified dentilisin has the ability to degrade plasma FN (75) and *T. denticola* challenge resulted in FN degradation in fibroblasts (36), suggesting that dentilisin directly degrades cellular FN. However, Miao et al. reported that *T. denticola* dentilisin did not directly degrade cellular FN and that dentilisin induced MMP-2-dependent FN fragmentation in periodontal ligament cells. The MMP-2-dependent FN fragmentation pattern induced by dentilisin activity resembles the FN fragmentation pattern seen in gingival crevicular fluid from periodontal disease lesions. This suggests that the distinction between plasma and cellular FN may be very important in terms of how *T. denticola* and other oral microbes interact with this protein that is essentially ubiquitous in the subgingival environment.

Laminin and collagen

T. denticola is reported to bind laminin and collagen (62, 76, 77), and this has been proposed as a mechanism of adherence to cells and ECM in the oral cavity. *T. denticola* Msp (22, 66, 67) and OppA (68) bind laminin. Recombinant Msp binds type I collagen (66), and Msp blocks phagocytosis of collagen by fibroblasts (47). It is not clear whether this last phenomenon is due to Msp competing for collagen or to Msp disruption of fibroblast membrane function. Dentilisin activity has been reported to either directly degrade (75) or indirectly induce degradation (78) of these substrates. The few *in vitro* studies in the literature do not clearly establish the biological role of *T. denticola* binding and degradation of these substrates. Further studies are required to determine the significance of interactions of *T. denticola* with these ECM components in the subgingival environment.

Glycosoaminoglycans

Hyaluronan (HA) is a high molecular weight glycosoaminoglycan prevalent in ECM and connective tissue. The potential role of HA as a substrate for *T. denticola* adherence is supported by two studies employing distinctly different approaches. Edwards et al. demonstrated the ability of rMsp to bind to several immobilized substrates including HA (66). Haapasalo et al. reported *T. denticola* binding to immobilized HA and showed that HA blocked binding of *T. denticola* to HA-coated beads (79). Interestingly, inhibition of dentilisin activity also blocked HA binding by *T. denticola* cells, suggesting a role for dentilisin in HA binding. To date, these studies

have not been followed up by analysis of isogenic mutants and purified native proteins.

Hyaluronidase-mediated degradation of HA increases the permeability of connective tissues and decreases the viscosity of body fluids (80). HA undergoes a high turnover rate, and HA degradation products act as an immune regulator, signaling through TLR2, TLR4, or both TLR2 and TLR4 in macrophages and dendritic cells (81). Hyaluronidases of pathogenic *Staphylococcus*, *Streptococcus*, and *Clostridium* spp. (among others) facilitate bacterial penetration through host tissues, and cell-bound HA serves as an antigenic disguise that prevents the recognition of bacteria by phagocytes (82). The ability to bind HA is an obvious prerequisite for HA degradation, and bacterial hyaluronidases generally also have HA-binding activity, but *T. denticola* interaction with HA has not yet been studied in detail. At least three species of cultivable oral spirochetes, including *T. denticola* produce a surface-expressed enzyme that hydrolyzes both HA and chondroitin sulfate (83). The 59-kDa enzyme purified from *T. denticola* (designated 'HA/CSase') hydrolyzed both of these ECM glycosoaminoglycans. Scott et al. suggested that this enzyme could have an active role in connective tissue degradation in periodontal disease (83). Analysis of the *T. denticola* genome suggests that this enzyme is encoded by TDE0471, predicted to encode a lipoprotein with sialidase activity. The relationship between this activity and the reported *T. denticola* HA binding activity is not known. The single study reporting HA binding also noted that there was no significant attachment of *T. denticola* to chondroitin-4-sulfate or dermatan sulfate (79).

Interactions with serum components

Nutrient acquisition

Routine growth of *T. denticola* requires serum supplementation of the complex medium formulation (84). Although a serum-free chemically defined medium has been reported, to date it has only been used in a single study of *T. denticola* fatty acid synthesis, primarily due to the technical challenge of its formulation (85). As an indication of the complexity of this medium, a commercial supplier has quoted a closely related formulation (OMIZ-P4 (86); ATCC Medium 2,131 (http://www.atcc.org)) at a price of well over US$1,000 per liter (J.C. Fenno, personal communication). Growth of *T. denticola* in a serum-containing medium is dependent on serum or albumin concentration (87). SDS-PAGE analysis of serum-containing culture medium during growth of *T. denticola* shows dentilisin-dependent degradation of albumin (J.C. Fenno, unpublished results).

Although *T. denticola* exhibits some chemotactic behavior toward glucose (88), it only weakly metabolizes glucose while preferentially deriving energy from amino acid fermentation (89). Consistent with its reliance on amino acids and peptides as primary nutrients, the *T. denticola* genome encodes eight ABC-type peptide uptake systems (25), presumably with a variety of peptide substrate specificities. To date, only one of these, encoded by OppA-F, has been characterized (68). OppA, the substrate binding protein of this system, is a 70-kDa outer membrane lipoprotein that binds FN, laminin, and plasminogen. The specific peptide substrate(s) taken up through OppA-F have not been determined, and an isogenic *oppA* mutant showed no defect in growth in culture. Several toxic peptide analogs specific for particular peptide uptake systems were tested and showed no difference in activity against wild-type and *oppA* mutant *T. denticola* (68). These results are most likely a reflection of the multiple redundant mechanisms of peptide acquisition in this organism.

Among cultivable oral organisms, *T. denticola* and members of the 'black pigmented' *Bacteroidetes*, including *P. gingivalis* produce the highest levels of hydrogen sulfide (H_2S) (90), a cytotoxic compound found in elevated levels in periodontal lesions (91). Metabolism of glutathione by *T. denticola* produces H_2S that is believed to play a role in the host tissue destruction seen in periodontitis (92). Recent work demonstrates that H_2S induces apoptosis in oral epithelial cells (93) as well as gingival fibroblasts and periodontal ligament cells (94). In addition to the production of a toxic metabolite from a host-supplied nutrient (glutathione), the enzyme that catalyzes the final step in the glutathione metabolism pathway is cystalyisin, a cysteine desulfhydrase that was originally characterized due to its hemolytic activity (95).

Fibrinogen

The ability of bacterial pathogens to bind fibrinogen is linked to pathogenic behavior of several bacterial species, especially those such as *staphylococci* and streptococci involved in endocarditis (96). Fibrinogen is a plasma protein that is essential for wound healing and also plays a role in tissue homeostasis (reviewed in (97)). Several investigators have documented the ability of dentilisin activity to hydrolyze fibrinogen (23, 98–100) Bamford et al. reported purification of the native dentilisin complex (CTLP) by fibrinogen affinity chromatography (100). Although fibrinogen binding and degradation was generally interpreted as an example of *T. denticola* general proteolytic activity toward potential nutrients in the host environment, it was also recognized as a potential virulence behavior that could contribute to the dysregulation of tissue homeostasis characteristic of periodontal disease. Fibrinogen binding by the Msp in both native and recombinant forms has been reported in studies using *T. denticola* cells, native Msp, and recombinant Msp (22, 66, 76). Although no studies have reported the fibrinogen binding activity of *msp*-deficient

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T. denticola, results of recent work by Bamford et al. strongly imply that Msp is not the only fibrinogen-binding protein of *T. denticola* (70). This study used fibrinogen affinity chromatography to purify the native dentilisin protease complex from *T. denticola* extracts and subsequently demonstrated the hydrolytic activity of the purified protease toward fibrinogen. *T. denticola* exhibited saturable binding kinetics to soluble and immobilized fibrinogen. Because *T. denticola* thrives in periodontal lesions harboring damaged tissue, the ability to interact with fibrinogen may contribute both to colonization and environmental modulation by the spirochete.

Environmental signaling and chemotaxis

All microorganisms sense and respond to changes in their environment, especially for purposes of nutrient acquisition. Studies of chemotaxis pathways have progressed in *T. denticola*, both before and after publication of the genome. Studies by Shi and others characterized chemotaxis-related gene expression (101, 102) and chemotactic behavior toward potential nutrients (88) and the requirement for chemotaxis function for penetration of a model epithelium (103). These studies confirmed the expected important role of environmental sensing for this highly motile organism. The mechanism of environmental sensing typically involves one or more two-component systems consisting of a sensor kinase and a response regulator. A recent study characterized AtcS-AtcR, the first growth phase-regulated two-component system, identified in the *T. denticola* genome sequence (104). Expression of this system in *T. denticola* responds to environmental conditions, and likely functions to regulate expression of appropriate metabolic or biosynthetic pathways as nutrients are depleted. This and other signaling and regulatory systems are the subject of a recent review by Frederick et al. (105). In another study of potential importance to environmental responses, Gonzalez et al. (106) characterized the phosphoenolpyruvate-dependent phosphotransferase system of *T. denticola*. The authors reported that, because *T. denticola* lacks PTS permeases normally utilized by bacteria for the uptake of simple carbohydrates, it is likely that the *Treponema* phosphoryl-transfer chain has unique modes of signal detection and sensory transmission. The role of this phosphotransferase system in *T. denticola* metabolism or environmental sensing remains unknown.

Modulation of host immune response pathways

Modulation of host innate immune response pathways contributes to pathology of both chronic local infections and life-threatening systemic inflammatory conditions that may be induced or exacerbated by chronic bacterial challenge (107). The early literature on innate and acquired immune responses to oral spirochetes (reviewed in (108)) contains limited analyses of effects of specific antigens, and some apparently contradictory reports. More recent work strongly implicates *T. denticola* outer membrane protein, including the PrtP protease complex (dentilisin) and Msp in these processes (53, 109, 110). Cytopathic and immunomodulatory effects of these protein complexes are under study by several groups. PrtP (dentilisin) is one of only two known lipoproteins in the subtilase family, the other being SphB1, a subtilisin-autotransporter that catalyzes maturation of the virulence factor FhaB (filamentous hemagglutinin) at the surface of *Bordetella pertussis* (111). The *T. denticola* protease locus, including *prcB, prcA,* and *prtP*, is conserved in several oral *Treponema* spp. (112–114). Studies with *T. denticola* mutants implicate PrtP protease activity in modulation of complement regulatory protein Factor H activity (115), activation of MMP-2 (71), and disruption of intercellular junctions (13). Studies with wild-type strains suggest involvement of dentilisin in cell adherence and cytotoxicity (40, 116), FN degradation (12, 36), and modulation of IL-8 expression (53). Both protein complexes exhibit adhesin-like binding activities (22, 116–118), induce cytoskeletal rearrangements, (12, 46) and are cytotoxic to epithelial cells (12, 40). Msp (but not PrtP) is hemolytic (40) and disrupts intracellular calcium regulation (50). Msp disrupts cytoskeletal actin in both gingival fibroblasts and neutrophils (46, 119). PrtP protease activity degrades ECM components as well as IL-8 (53, 54), IL-1ß, IL-6, TNF-α (120), and other bioactive molecules involved in inflammatory vascular responses (98, 121).

Resistance to serum killing

The ability of periodontal organisms to survive the bactericidal activity present in serum and gingival crevicular fluid is suggestive of a specific mechanism of complement resistance. Although some evidence supports a role for the *T. denticola* surface protease dentilisin (CTLP) in serum resistance, due to its ability to hydrolyze α-chain of C3, producing iC3b (122), recent studies implicate a Factor H-binding lipoprotein in this behavior (115, 123). Because most spirochetal Factor H-binding proteins are acylated but otherwise relatively unconserved, this work utilized a genome- and proteomics-based approach to identify nine candidate genes encoding small lipoproteins containing coiled-coil domains (123). One of these (FhbB, encoded by TDE0108) bound Factor H to the cell surface, and the greatly increased susceptibility of an isogenic *fhbB* mutant to serum killing indicates that FhbB is primarily responsible for *T. denticola* complement resistance (124). Interestingly, the PrtP protease also plays a role in FhbB-mediated Factor H binding. Factor H bound to the *T. denticola* surface is cleaved by dentilisin activity to a size that closely approximates FHL-1 (115). However, as there was no difference in complement resistance between parent

and *prtP*-deficient *T. denticola* strains (124), the precise role of dentilisin in interaction with the complement pathway remains to be determined.

Resistance to antimicrobial peptides

Antimicrobial peptides with a wide range of structure, charge, and amino acid content comprise a significant component of innate immunity. Several cell types associated with mucosal surfaces produce small ($<$6-kDa) cationic peptides, designated β-defensins (hBDs), that are active against a wide range of microbes. Antimicrobial activity of defensins is believed to be due to disruption of the bacterial cytoplasmic membrane, although the exact mechanism of action remains somewhat controversial (125). While defensin susceptibility of periodontal organisms varies with the particular defensin molecule, bacterial species and bacterial strain, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Fusobacterium nucleatum* generally exhibit at least partial sensitivity to hBDs 1, 2, or 3 (126). In contrast, Brissette et al. reported that *T. denticola* is resistant to hBDs 2 and 3 (127), as are several oral *Treponema* spp. (128). The mechanism of resistance is not yet understood, but several mechanisms that contribute to resistance in other organisms have been ruled out, including dentilisin proteolytic activity, ABC efflux transporters, and competition with other host proteins for binding sites (129). Potential mechanisms of *Treponema* resistance to hBDs include proton motive force-driven efflux and reduced binding affinity due to absence of lipopolysaccharides (LPS) on the outer membrane of the cell. In any case, this striking insensitivity to killing effects of hBDs may contribute to the abundance of treponemes in periodontal disease lesions.

Degradation of immunoglobulins

The ability to hydrolyze immunoglobulins is a potential mechanism of evasion of adaptive immune responses. Although a number of microbial pathogens possess specific proteases with specific activity against immunoglobulins, *T. denticola's* ability to degrade immunoglobulins *in vivo* has been somewhat controversial. While dentilisin has demonstrated *in vitro* activity against IgG (23, 130), Hollmann and Van der Hoeven reported that intact *T. denticola* did not degrade IgA, IgG, or albumin (131). It is reasonable to question these results in light of the dentilisin studies and given the fact that serum albumin is a preferred nutrient source of *T. denticola* (87).

In a recent study, Ishihara et al. characterized IdeT, a *T. denticola* homolog of IdeS, that is an IgG-specific streptococcal protease (132). The C-terminal domain of the *T. denticola* protein (designated 'dentipain') is a protease that is cleaved and secreted into the extracellular environment. An isogenic mutant strain in which the dentipain domain was deleted was significantly less virulent in a mouse abscess model, suggesting that IdeT contributes to *T. denticola* virulence (133). Further studies are required to characterize the role of IdeT in interaction with immunoglobulins and other serum proteins in the subgingival environment.

Lipooligosaccharide (LOS) and lipoprotein effects

The *T. denticola* genome lacks an identifiable LPS synthesis pathway (25). Biochemical analysis of the *T. denticola* outer membrane revealed the presence of a novel glycolipid or lipooligosaccharide (LOS) that comprises the outer leaflet of the outer membrane bilayer (134). This LOS, although similar in overall structure and function to the lipopolysaccharide present on most Gram-negative organisms, has a distinctly different pattern of sugar molecules and lacks the lipid A component of a typical LPS and is also biochemically distinct from lipoteichoic acid. Classical LPS proinflammatory signaling is primarily through TLR4, with some involvement of TLR2 in many cases. However, in spirochetes, many of which lack LPS, lipoprotein-mediated signaling through TLR2 is a primary activator of inflammatory responses (51, 135). Rosen et al. reported activation of murine macrophages by both LOS and delipidated lipoproteins of *T. denticola* (56), but did not identify signaling pathways involved. Recently, the same group reported that LOS induced macrophage responses through a TLR4-MyD88 pathway, whereas the major outer membrane protein Msp induced innate immune responses through TLR2-MyD88 (136). It should be noted that Msp is not acylated, so the mechanism of activation may be distinct in this case. In closely related treponemes, *T. maltophilum* LOS activation of NF-κB was reported to be dependent on TLR2, while both TLR2 and TLR4 were involved in activation of innate immune responses to *T. brennaborense* LOS (137, 138). Other recent studies focused on downstream effects of likely TLR-mediated signaling events. Choi et al. reported that *T. denticola* LOS induced osteoclastogenesis and MMP-9 expression in cultured murine osteoblasts and suggested that these processes may be contributory mechanisms to bone loss associated with periodontal diseases (139). A similar study that focused on gingival fibroblasts reported significantly increased MCP-1, IL-8, PGE2, and MMP-3 in LOS-challenged fibroblasts.

Summary

Treponema denticola, the most readily cultivable oral spirochete, is the model organism for the study of spirochete–host interactions in periodontal disease. Research in recent years has greatly expanded our understanding of both the biology of spirochetes and the mechanisms utilized by these opportunistic commensal pathogens to both survive as members of the 'normal' oral flora as well as to participate in the induction of

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periodontal tissue destruction and thrive in the resulting environment. Numerous potentially pathogenic behaviors and potential virulence factors were identified for some of these behaviors, but it is rather striking that two multifunctional outer membrane protein complexes were reported to both directly and indirectly mediate cellular damage participate in many of the identified pathogenic behaviors of this organism. The surface-expressed PrtP lipoprotein protease complex (dentilisin) and the oligomeric Msp are among the most studied *T. denticola* virulence determinants. While this may overshadow the potential importance of other as yet poorly documented behaviors, it is evident that Msp and dentilisin are key players in *T. denticola* periodontal pathogenicity. Besides functioning in nutrient acquisition, dentilisin contributes to tissue penetration and cleaves several proteins involved in innate and adaptive immunity, likely favoring dysregulation of tissue homeostasis. Expression of dentilisin is required for native expression of the major surface protein (Msp) that binds FN, has cytotoxic pore-forming activity, and disrupts intracellular calcium responses. A deeper understanding of the roles of these and other *T. denticola* proteins in microbe–host interactions requires taking advantage of recent advances in spirochete molecular biology to analyze their cytopathic and immunomodulatory behaviors.

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Conflict of interest and funding

There is no conflict of interest in the present study for the author.

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