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ORIGINAL PAPER

Use of PCR to detect *Entamoeba gingivalis* in diseased gingival pockets and demonstrate its absence in healthy gingival sites

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Abstract Investigators using light microscopy have identified the protozoan parasite *Entamoeba gingivalis* from diseased gingival pockets for nearly 100 years. The objective of the present investigation was to develop a molecular biology approach for determining the presence of *E. gingivalis* in both diseased gingival pockets and healthy gingival sites. For this, a previously developed conventional polymerase chain reaction (PCR) was evaluated and a real-time polymerase chain reaction assay was developed. Paper points were inserted into the base of the sulcus of both diseased gingival pockets and healthy gingival sites. DNA was extracted using the QIAamp DNA mini kit, and subsequently analyzed using conventional and real-time PCR analysis. A previously described primer set specific for the small subunit ribosomal RNA gene (SSU rDNA) of *E. gingivalis* was used for the conventional PCR. For the real-time PCR, a primer set was designed to amplify a 135-bp fragment inside the SSU rDNA of *E. gingivalis*. A conventional PCR assay detected *E. gingivalis* in 27% of diseased gingival pockets. The real-time PCR using a different primer set detected protozoa in 69% of diseased pocket sites. Thus, the latter technique proved more sensitive for detection of *E. gingivalis*. No *E. gingivalis* were detected in any of the healthy gingival pocket sites using either type of PCR assay. Results support a concept that the presence of *E. gingivalis* is associated only with diseased gingival pocket sites. The newly described methodology may also serve to provide a novel eukaryotic cell marker of disease status in gingival pockets.

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Introduction

Periodontitis with its various clinical forms represents one of the most widely distributed types of oral disease. Approximately 5% to 20% of any population is affected by severe generalized periodontitis (Burt 2005). This inflammatory condition is associated with a chronic bacterial infection caused by anaerobic Gram-negative bacteria (Armitage 1999; Haake et al. 2006; Socransky 1977). For nearly 100 years, light microscopic studies have also demonstrated a high incidence of the protozoan parasite *Entamoeba gingivalis* in individuals suffering from oral disease including periodontitis (Bass and Johns 1915; Barrett 1914). This has led to a speculation that it might also be a contributing factor to periodontal disease. More recently, *E. gingivalis* was identified in all 65 subjects with destructive periodontitis, but was absent in individuals with marginal gingivitis or in excellent periodontal health (Keyes and Rams 1983). Similarly, in 1989 a clinical survey of *E. gingivalis* by multiple sampling in patients with advanced periodontal disease revealed the occurrence of *E. gingivalis* in all ten periodontal patients tested (Linke et al. 1989). However, the average protozoa prevalence (based on microscopic observation) was 62% (62 samplings were positive from a total of 100 samplings). Conversely, on occasion, some have reported the general presence of *E.*

gingivalis in disease-free individuals (Dao et al. 1983) and in part to be age dependent and related to the amount of calculus present on teeth (Wantland and Laurer 1970). In 1983, it was also reported that there was an association between oral deterioration and *E. gingivalis*. After recovery of *E. gingivalis* from apparently healthy tissue, there would be periodontal decline unless the protozoa were eradicated in the meantime (Lyons et al. 1983). One may also encounter difficulty in identifying the protozoa (Krogstad et al. 1978) and it can be difficult to differentiate *E. gingivalis* from a macrophage (Dao 1985).

In the past decade, newer molecular biology-based identification techniques such as the polymerase chain reaction (PCR) have been successfully used to identify bacteria in the gingival pockets of patients suffering from periodontal disease (Ashimoto et al. 1996; Jervoe-Storm et al. 2005). The PCR can also document the presence of the potential bacterial pathogens before, during, and after disease treatment, and this technology is commercially available to dental practice activities (http://www.hain-lifescience.de/en/products/microbiology.html.).

The development and application of this newer technology to monitor the presence of *E. gingivalis* in periodontitis and its treatment is lacking. Based on light microscopy, there is the well documented close association of this parasite with periodontal disease and its potential to contribute to the condition. Therefore, it might be prudent to develop and use molecular biology methodology such as the PCR to detect and quantify the occurrence of *E. gingivalis* in individuals with periodontal disease. In 1996, DNA oligonucleotides were described that target the small subunit ribosomal RNA gene (SSU rDNA) of *E. gingivalis*, and they were used in the PCR to amplify the DNA of laboratory cultures of *E. gingivalis* (Kikuta et al. 1996). The primers also amplified DNA from subgingival plaque samples of patients.

In the current study, a primer set was successfully used to identify the occurrence of protozoa in gingival pockets of patients diagnosed with periodontal disease. We failed to detect *E. gingivalis* not only in the gingival tissue of healthy patients, but also within healthy gingival pocket sites of diseased patients. Additional insight into a close association between the presence of *E. gingivalis* and periodontitis was supported by development of a real-time PCR assay that allowed for a more sensitive specific detection and quantification of the parasite. *E. gingivalis* is morphologically indistinguishable from *Entamoeba histolytica* (Dao 1985) and can cause a diagnostic problem if found in the sputum of patients studied for pulmonary masses (Dao et al. 1983). Development of a real-time PCR assay for *E. histolytica* was reported in 2005 (Roy et al. 2005).

Here we report the development of a real-time PCR assay for *E. gingivalis* that was more sensitive than the conventional PCR assay for detection of this amoeba. Results of this study further demonstrated that use of molecular biology techniques for detection of *E. gingivalis* may serve to provide a novel eukaryotic cell marker of disease status in gingival pockets. The results also provide a means to further help identify a potential role for this organism in periodontal disease.

Materials and methods

Sampling methodology

The study was in accordance with compliance policy at Middle Tennessee State University. Subjects for the study were recruited from the Advanced Institute for Oral Health (Brentwood, TN). Patients were screened, examined, and selected for participation if they met criteria for sites of periodontal disease and good oral health. Only previously untreated patients presenting with periodontal disease were included in this study. Patients with a history of systemic antibiotic usage within the previous 6 weeks were excluded. Patient sample sites were organized into one of three categories: destructive periodontitis (gingival pockets ≥7 mm, marginal periodontitis (gingival pockets >4 mm), and healthy (gingival pockets <3 mm).

Prior to sampling, the supragingival plaque was removed with a sterile curette, and the sample site was dried with a sterile cotton roll. Sampling of diseased sites was performed prior to mechanical treatment of the pocket. A pair of sterile forceps was used to insert one paper point at a time down to the base of the sulcus. The area which occurs between the tooth and gingiva (gum) and the gum tissue that surrounds the tooth was considered the sulcus (an unusually deep gingival sulcus was considered a periodontal pocket). Samples were collected according to procedures used for PCR detection of bacteria periodontal pathogens from the sulcus (Micro-IDent, Hain Lifesciences, Nehren, Germany). The paper point was transferred into a microcentrifuge tube and assigned a corresponding patient letter. The collection procedure was performed once for detection of protozoa and once for bacteria detection. Samples were stored at −80°C until the DNA extraction was completed. This procedure was completed twice for most patients with the second sample (if taken) sent to Hain Lifesciences (Nehren, Germany) for the Micro-IDent test to detect and quantify bacteria. Samples from disease-free (orally healthy) patients were collected in the same manner; however, no mechanical treatment was required.

Laboratory procedures

A vial of *E. gingivalis* (ATCC 30928) from the American Type Culture Collection (Manassas, VA, USA) was shipped

in TYGM-9 medium. Based on hemocytometer counts, the culture contained 9×10^4 amoebae trophozoites/mL (the vial of xenic *E. gingivalis* from ATCC contained concentrated trophozoites, personal communication). From this, twofold serial dilutions were prepared, and DNA extracted from each dilution to form a standard curve for the real-time PCR assay (Fig. 1).

DNA was extracted from paper points using the QIAamp DNA mini kit (QIAGEN, Hilden, GR) according to the manufacturer's instructions. Extracted DNA was used immediately or stored at −80°C until use. PCR products (15 μl each) were combined with 3 μl loading dye and separated in a 1% agarose gel that included ethidium bromide.

Conventional PCR assay

Primer sets EGO-1 and EGO-2 were used to amplify the SSU rDNA gene of *E. gingivalis* (Kikuta et al. 1996), 18SF and 18SR served as universal eukaryotic (for all eukaryotic cells) SSU rDNA primers (Zhang et al. 2004), and 8F and 1540R served as universal bacterial SSU rDNA primers (Fields et al. 2005). The latter two primer sets were used as positive controls to ensure PCR inhibitors were not present in the samples. All of the samples were positive for eukaryotic and bacterial SSU rDNA, which was used as an indication of the absence of PCR inhibitors. Each 25-μl PCR reaction consisted of 2.5 μl of extracted patient DNA, 2.5 μl of the forward and reverse primers at final concentrations of 0.2 μM each, 17.5 μl of distilled water, and one PuRe Taq Ready-To-Go PCR bead (2.5 units of PuRE Taq DNA polymerase, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 mM of each dNTP), stabilizers, and bovine serum albumin (GE Healthcare, Piscataway, NJ).

Real-time PCR assay

Oligonucleotide primers were designed using software from IDT Scitools (Integrated DNA Technologies, Coralville, IA). The primer pair for real-time PCR (Table 1) specifically amplified a 135-bp fragment inside the SSU rDNA of *E. gingivalis* (GenBank accession number D28490). The primers were purchased from Integrated DNA Technologies. Each 25-μl reaction consisted of 2.5 μl of patient DNA, 2.5 μl of the forward and reverse primer, 5 μl of distilled water, and 12.5 μl of Bio-Rad (Hercules, CA) iQ SYBR Green Supermix (25 units iTaq DNA polymerase, 3 mM $MgCl_2$, dNTPs, SYBR Green I, 10 nM fluorescein, buffer, and stabilizers). The *E. gingivalis* SSU rDNA was amplified in a Bio-Rad iQ5 optical system with software version 1.0 under the following conditions: 95°C for 7.5 min, 40 cycles of 95°C for 1 min, 47°C for 30 s, 72°C for 30 s, followed by a 5-min extension at 72°C. A melt curve was included to ensure that only one product was amplified. The primers did not amplify products from other eukaryotic or bacteria cells tested and melt curve analyses performed for all reactions confirmed that the melt curves matched that of the *E. gingivalis* DNA control. The melt curve began at 95°C for 5 min followed by a 0.5°C decrease in temperature every 30 s to 47°C.

Samples were evaluated as positive for the presence of *E. gingivalis* DNA if fluorescence values crossed the cycle threshold (C_T) by 35 amplicon cycles (Fig.1). The C_T value represents the PCR cycle at which fluorescence, and thus DNA, is logarithmically increasing in the reaction. The earlier the fluorescence crosses the C_T, that is the lower the C_T value, the greater the amount of DNA present in the sample. All samples were run in triplicate (as a single replicate). A single plot of C_T value vs the number of *E. gingivalis* trophozoites was made with all dilutions performed in triplicate and the C_T values were averaged. All values that did not cross the C_T were designated as negative.

Micro-IDent bacteria detection and quantification

In some patients (18 of the 26 patients), duplicate paper point samples from diseased gingival pockets were sent to Hain Lifescience (Nehren, Germany). DNA was extracted for PCR and it was used to detect and quantify a variety of anaerobic bacteria associated with periodontal disease.

Results

PCR analysis was used to determine the occurrence of *E. gingivalis* in a diseased gingival pocket for 26 individuals diagnosed with periodontal disease. A gingival pocket from disease-free individuals was analyzed also for comparative purposes. Using conventional PCR methodology, 7 of 26 (27%) diseased pocket sites tested positive for *E. gingivalis* based on the presence of a 1.4-kb PCR product (Fig. 2). Subsequently, DNA extracted from the same pockets was tested for the presence of *E. gingivalis* using real-time PCR methodology with a different primer set. Using this assay, 18 of the 26 (69%) diseased pocket sites tested positive based on the presence of a 135-bp real-time product. Comparisons of the conventional and real-time PCR results are summarized in Table 2. PCR analysis (both a conventional and real-time PCR) was also used to determine the occurrence *of E. gingivalis* in healthy gingival pockets from five disease-free individuals and in healthy gingival pocket sites of seven patients diagnosed with periodontal disease. No *E. gingivalis* were detected in any of the 12 healthy gingival pocket sites tested.

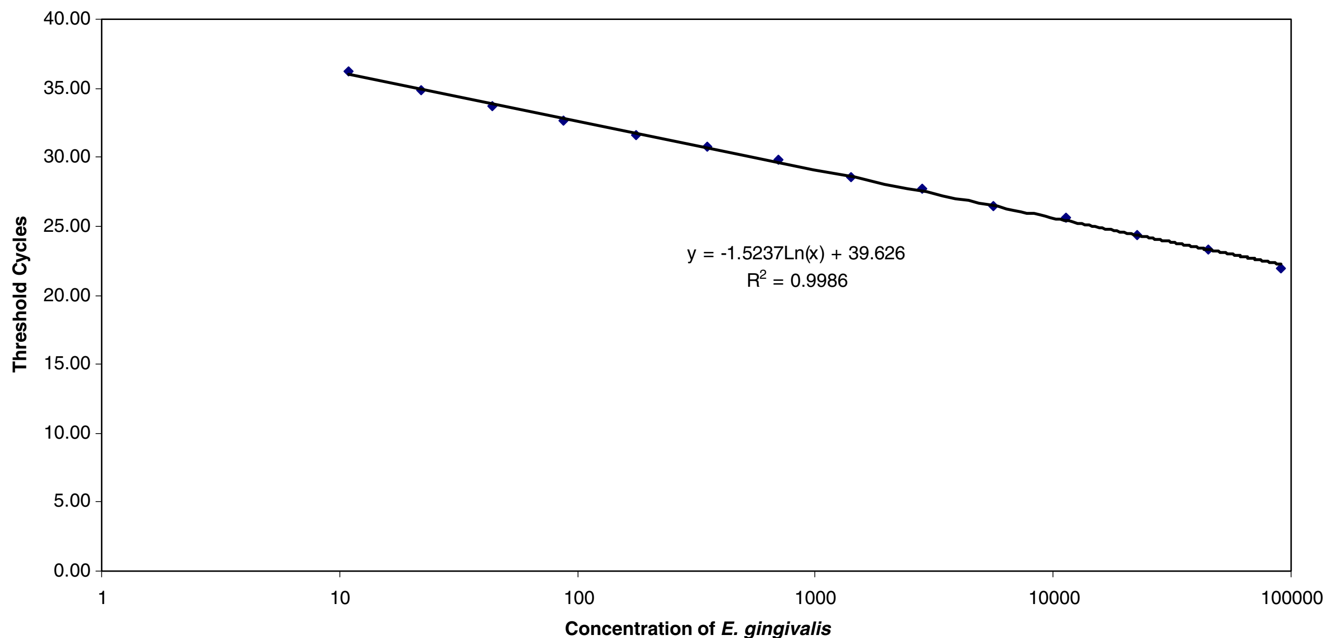

Fig. 1 The plotted C_T value for extracted DNA from twofold serial dilutions of *E. gingivalis* trophozoites. Using a C_T value of 35 as the upper limit of detection, as few as 20 trophozoites can be detected by real-time PCR

The Micro-IDent bacteria detection and quantification provided information on the potential occurrence of 11 different genera of bacteria. *Fusobacterium nucleatum/periodonticium* was reported from the diseased pocket of all of the 18 patients tested for bacteria (Table 3). The presence and relative number of the other genera of bacteria varied from one patient to another without distinct association with *E. gingivalis* (ANOVA using coded data; $P<0.0001$).

Discussion

Among the *Entamoeba* species, only *E. histolytica* is a well-documented human pathogen. The ability to document other *Entamoeba* species at diseased human sites requires accurate and efficient detection methodology. The potential to use the PCR for detection of oral amoebae that might contribute to disease development is supported by commercially available PCR technology to detect periopathogenic bacteria in the gingival sulcus (Micro-IDent, Hain Lifesciences). Riboprinting analysis has revealed phylogenetic relationships in the genus *Entamoeba* (Clark and Diamond 1997). It joined restriction site polymorphism analysis and rDNA amplification to determine sequence variation in SSU rDNA and was initially described by this term in 1991 (Clark and Diamond 1991). Two *E. gingivalis* oral isolates had the same riboprint pattern and one of these (ATCC 30928) was the same strain used in the current

Table 1 Primer sets for conventional and real-time PCR

Primer set	Sequence	Product size (bp)	Purpose
18SF	5′-GCTTGTCTCAAAGATTAAGCCATGC	1,800–2,000	Eukaryotic SSU rDNA[a] (positive control)
18SR	5′-CACCTACGGAAACCTTGTTACGAC		
8F	5′-AGAGTTTGATCCTGGCTCAG	1,500	Bacterial SSU rDNA[a] (positive control)
1540R	5′-AAGGAGGTGWTCCARCCGCA		
EGO-1	5′-GAATAGGCGCATTTCGAACAGG	1,400	*E. gingivalis* SSU rDNA[a]
EGO-2	5′-TCCCACTAGTAAGGTACTTACTC		
EGHF	5′-TACCATACAAGGAATAGCTTTGTGAATAA	135	*E. gingivalis* SSU rDNA[b]
EGHR	5′-ACAATTGTAAATTTGTTCTTTTTCT		

SSU rDNA small subunit ribosomal RNA

[a] Used in conventional PCR

[b] Used in real-time PCR

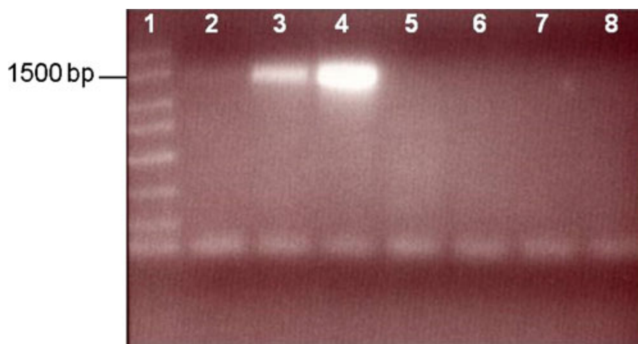

Fig. 2 Amplification of a 1.4 kb PCR product from an *E. gingivalis* laboratory culture and a positive patient sample from a diseased gingival pocket. *Lane 1* is the standard ladder (PCR marker, Sigma-Aldrich, St. Louis, MO). *Lane 2* is a negative control (water). *Lane 3* is a positive control (*E. gingivalis* from approximately 500 trophozoites of an ATCC culture). *Lane 4* is a positive patient sample, and *lanes 5–8* are negative results from patients

study. A variant riboprint was observed in an *E. gingivalis* (ATCC 30956) isolated from a uterine infection. Additionally, the oral isolates may have two forms of the SSU-rRNA gene. Thus, two ribodemes exist within *E. gingivalis* (Clark and Diamond 1997). It was concluded, however, that plural ribodemes within a species did not merit recognition as distinct species. These differences likely are not a reflection of different habitats. Rather, riboprinting suggested the presence of variant genes within an individual *E. gingivalis* isolate. Riboprinting, however, will miss a significant amount of sequence variation and the genetic distances between *Entamoeba* species based on riboprinting are large.

The role of this potential sequence variation in PCR-based detection methodology remains to be clearly defined. A previous investigation and the current study using clinical specimens support the potential of PCR technology for use to identify *E. gingivalis* in diseased clinical samples. The results for the real-time PCR increased sensitivity suggest this might be of greater preference over the conventional PCR.

A conventional PCR assay that amplified DNA from laboratory cultures of *E. gingivalis* and clinical samples has been previously reported (Kikuta et al. 1996). When tested against nine other species of protists (including *Entamoeba* species), ten species of oral bacteria, and human leukocytes, no PCR product of any length was produced. Thus, the primers used (EGO-1 and EGO-2) were considered specific for *E. gingivalis*. Subsequent PCR testing of subgingival plaque samples (one curette scrape) from eight patients with marginal periodontitis or gingivitis resulted in an amplification product in two samples. No specific DNA amplification occurred from 20 supragingival samples from healthy humans. It was concluded that the EGO-1 and EGO-2 primers detected as few as 30 *E. gingivalis* cells in reaction mixtures and could be applicable to clinical use (Kikuta et al. 1996). In this report, 6.25% (2 of 32 subgingival plaque samples) of patient samples proved positive by PCR analysis.

For the present study, the EGO-1 and EGO-2 primer sets were used to detect *E. gingivalis* in patients with periodontal disease. Here, 27% (7 of 26 diseased pocket sites) tested positive. No positives occurred from healthy gingival pockets (even healthy gingival pocket sites within patients suffering from periodontal disease). This further supports a concept that with conventional PCR analysis, *E. gingivalis* specifically associated with the presence of periodontal disease. The higher incidence (percent positive) of positives samples in the current study could be a reflection of differences in sample collection. In the study by Kikuta et al. (1996), subgingival plaque samples were composed of one curette scrape dispensed in 100 μl. In the present study, samples for PCR testing consisted of insertion of a paper point to the base of the sulcus for sample recovery.

For the present study, the authors successfully modified the conventional PCR assay to detect *E. gingivalis* in patients with periodontal disease. Subsequently, a real-time PCR assay was developed. Advantages of real-time PCR over conventional PCR are that it is performed in a closed system where post-PCR handling is not required, it is highly sensitive, and can be used for quantitative purposes. Since more patients were found to be positive for *E. gingivalis* with the real-time PCR assay, it should be considered to be more sensitive for the detection of *E. gingivalis*. No *E. gingivalis* were detected when healthy gingival pocket sites from periodontal disease patients were tested by conventional or real-time PCR. Additionally, no *E. gingivalis* were detected from gingival pocket sites from individuals identified as having good oral health. Collectively, the real-time PCR results also supported the concept that *E. gingivalis* were specifically associated with periodontal disease.

The possibility that *E. gingivalis* might elaborate proteolytic enzymes that could contribute to the pathogenesis of periodontitis is not a novel hypothesis (Gottlier and

Table 2 Comparison of the conventional PCR and real-time PCR for detection of *E. gingivalis* in a diseased gingival pocket from 26 patients diagnosed with periodontal disease

	Positive	Negative
Conventional	7	19
Real-time PCR[a]	18[a]	8

PCR polymerase chain reaction

[a] All real-time PCR patient samples were also positive by conventional PCR

Table 3 Micro-IDent detection and quantification of bacteria present in 18 different patients with *Entamoeba gingivalis*

Patient	Aa	Pg	Tf	Td	Pi	Pm	Fn	Cr	En	Ec	Cs
A	−	+	++	+	+	+	++	+	−	+	+++
B	−	++	++	+	−	+	++	++	−	(+)	(+)
C	++	−	−	−	−	+	++	−	−	−	(+)
D	−	+	++	+	+	−	++	−	−	++	(+)
E	−	(+)	++	(+)	−	(+)	++	−	++	−	(+)
K	−	−	+	−	−	(+)	++	(+)	−	+	−
L	−	−	−	+	−	(+)	+++	−	−	++	−
M	−	++	−	−	−	−	++	(+)	−	+	+
O	(+)	+	+	(+)	−	(+)	++	(+)	−	++	+
P	+++	++	++	+	−	(+)	++	+	(+)	+	(+)
Q	−	+	++	(+)	−	(+)	++	+	(+)	+	−
R	−	−	++	+	++	++	++	(+)	++	+	−
U	−	−	−	−	−	−	(+)	−	−	−	−
V	−	−	++	+	−	+	++	(+)	−	+	+
W	−	++	++	++	(+)	+	++	+	(+)	(+)	−
X	−	−	++	++	−	−	++	−	−	+	−
Y	−	++	+	−	−	(+)	++	−	−	−	−
Z	(+)	++	++	+	++	(+)	++	+	+	++	−

ANOVA showed significant differences between all 11 species ($P<0.0001$)

$-=<10^4$, $(+)=10^4$, $+=<10^5$, $++=<10^6$, $+++=>10^7$

Aa Actinobacillus actinomycetemcomitans, *Fn Fusobacterium nucleatum/periodonticum*, *Pg Porphyromonas gingivalis*, *Cr Campylobacter rectus*, *Tf Tannerella forsythia*, *En Eubacterium nodatum*, *Td Treponema denticola*, *Ec Eikenella corrodens*, *Pi Prevotella intermedia*, *Cs Capnocytophaga* sp. (*gingivalis, ochracea, sputigena*), *Pm Peptostreptococcus micros*

Miller 1971). Previous studies with the closely related *E. histolytica* have clearly identified virulence factors that contribute to and result in tissue destruction. Cysteine proteases serve as virulence factors and are important proteolytic enzymes in parasitic protozoa (Sajid and McKerrow 2002). *E. histolytica* contains 20 cysteine protease genes and orthologous sequences were also present in *Entamoeba dispar* (Bruchhaus et al. 2003; Tillack et al. 2007). Cysteine protease inhibitors greatly impacted the ability of *E. histolytica* to produce liver abscess development in laboratory animals (Li et al. 1995; Stanley et al 1995). If *E. gingivalis* possesses similar genes to express cysteine proteinases, it would suggest a new factor to consider in treating periodontal disease.

Additional virulence factors included the galactose/N-acetyl D-galactosamine-inhibitable (Gal/GalNAc) adherence lectin (Petri et al. 2002) and phospholipases (Ravidn 1986). Lipase increases were also associated with *E. histolytica* energy metabolism adaptation to the host intestinal environment (Gilchrist et al. 2006).

During the last 20 years, a variety of laboratory studies have demonstrated that some obligate and facultative intracellular bacteria pathogens benefit from associations with facultative pathogenic amoebae in the genera *Naegleria* and *Acanthamoeba*. Amoebae can serve as host cells for bacteria in the genera *Legionella*, *Parachlamydia*, *Listreria*, and other intracellular bacteria pathogens (Greub and Raoult 2004). Amoebae can also serve as host cells to protect bacteria from detrimental environmental factors. Interaction with amoebae may promote expression of virulence traits for *Legionella pneumophilia* and *Mycobacteria avium* (Cirillo et al. 1997; Cirillo et al. 1994). The likelihood bacteria could reside within cells of *Entamoeba* species were suggested in xenically (with associated intestinal flora) cultured *E. dispar* and *E. histolytica* (Pimenta et al. 2002). Several bacteria were always identified within vacuoles of the latter. In *E. dispar*, however, only single bacterium occurred in vacuoles and on occasion bacteria were free in the cell cytoplasm. Studies addressing potential interactions between periopathogenic bacteria and *E. gingivalis* are lacking. However, since both occur in diseased gingival pockets, interactions would be expected to occur. In one in vitro study, it was reported that *Actinobacillus actinomycetemcomitans* was affected by the presence of unidentified oral amoebae. The amoebae enhanced the growth of *A. actinomycetemcomitans* in media which otherwise failed to meet nutritional requirement (Derderian 1991). The bacteria were found around the exterior of amoebae and appeared to be localized in its vacuoles. Although amoebae consume bacteria as a food source, some bacteria may survive phagocytosis and multiply within amoebae (Greub and Raoult 2004). It was suggested that this interaction could be potentially significant since bacteria harbored inside amoebae could be protected from the immune system or antibiotics which are given as a part of therapy during periodontal treatment. In the absence of periodontal disease treatments which

might eliminate *E. gingivalis*, bacteria sheltered within amoebae could exit the amoebae to reseed the tissues or the sulcus and possibly create a refractory case (Derderian 1991).

There are several challenges ahead for determining the exact role of *E. gingivalis* in periodontal disease. The organism is extremely difficult to culture and it cannot be cultured in the absence of bacteria (Gannon and Linke 1992). Some bacteria accompanying the xenic cultures are detrimental to growth of *E. gingivalis* while other bacteria are beneficial to trophozoite growth (Gannon and Linke 1992). Additional insight into the potential roles of *E. gingivalis* in periodontal health could be addressed by using molecular biology techniques to identify potential virulence factors in clinical isolates and characterize its interactions with periopathogenic bacteria in laboratory studies using in situ hybridization to detect periopathogenic bacteria that might be localized in the amoebae.

Periodontal disease may be a reflection of the interplay between several etiological agents and environmental factors. Many antibiotics given to treat periodontal disease would have no effect on protozoa. Often, periodontists observe reductions in bacteria within the gingival socket, but the patient will still have symptoms that indicate active disease. A real-time PCR assay for *E. gingivalis* could measure parasite loads and determine if treatments are efficacious in elimination of amoebae. Results of this investigation also provide a framework to help assess a potential etiological role for *E. gingivalis* in periodontal disease.

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