Abstract  Periodontitis is considered a consequence of a pathogenic microbial infection at the periodontal site and host susceptibility factors. Periodontal research supports the association of Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, and Bacteroides forsythus, and periodontitis; however, causality has not been demonstrated. In pursuit of the etiology of periodontitis, we hypothesized that the intracellular bacteria Chlamydia trachomatis may play a role. As a first step, a cross-sectional study of dental school clinic patients with established periodontitis were assessed for the presence of C. trachomatis in the oral cavity, and in particular from the lining epithelium of periodontal sites. C. trachomatis was detected using a direct fluorescent monoclonal antibody (DFA) in oral specimens from 7% (6/87) of the patients. Four patients tested positive in specimens from the lining epithelium of diseased periodontal sites, one patient tested positive in healthy periodontal sites, and one patient tested positive in the general mucosal specimen. In conclusion, this study provides preliminary evidence of C. trachomatis in the periodontal sites. Planned studies include the use of a more precise periodontal epithelial cell collection device, the newer nucleic acid amplification techniques to detect C. trachomatis, and additional populations to determine the association of C. trachomatis and periodontitis.

Keywords  Chlamydia · Chlamydia trachomatis · Fluorescent antibody technique · Periodontal diseases · Periodontitis

Introduction

Periodontitis is characterized by apical migration of the periodontal attachment to the root surface and destruction of the proximal alveolar bone [12]. These diseases are generally considered a consequence of a pathogenic microbial infection at the periodontal site and host susceptibility factors [29]. The prevalence of severe periodontitis is estimated at 7% to 15% of adult populations [11].

In the search for the etiologic agent(s) of periodontitis, research has focused on the microbes of subgingival dental plaque. The relative interest in the microbial dental plaque can be estimated by the large quantity of published research concerning dental plaque and periodontitis. In contrast, there are fewer studies investigating the relationship between periodontitis and bacteria within the periodontal epithelium. In a MEDLINE search of articles from 1966–April 1998, at least 3,815 publications had the keywords “dental plaque” and “periodontitis.” By contrast, the keywords of “bacteria” and “epithelium” and “periodontitis” appeared in only 35 publications.

The Consensus Report, “Periodontal Diseases: Pathogenesis and Microbial Factors,” from the 1996 World Workshop on Periodontics lists evidence for the association of specific dental plaque microbes with various forms of periodontal disease [16]. The evidence for three microbes, Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, and Bacteroides forsythus, as etiologic agents is considered strong. The current viewpoint is that a
“periodontopathic bacterial flora is ‘necessary but not sufficient for disease’ or that periodontal diseases are ‘specific mixed infections which cause periodontal destruction in the appropriately susceptible host’” [52].

Our preliminary study pursued the etiology of periodontitis from the perspective of infection of the periodontal lining epithelium. “Periodontal lining epithelium” is used to describe the superficial epithelial lining the sulcus or pocket, and the junctional epithelium. This study targeted Chlamydia trachomatis which is known for infections of the epithelial cell linings of the eyelids (conjunctivitis and trachoma), respiratory tract (neonatal pneumonia) and urogenital tracts [7, 48, 49, 51, 62, 63]. Through review of the literature, similarities were noted between the natural history of chlamydial cervicitis and periodontitis. First, C. trachomatis preferentially infects the columnar or transitional epithelial cells lining the endocervix, and these were considered similar to the cuboidal or junctional epithelial cells lining the periodontal sulcus [8, 62, 73]. Second, both infections are characterized as chronic, usually asymptomatic, with probable bursts of activity, and with the tissue damage due in part to the host immune response [35, 45, 59, 69, 73]. Also, both infections are affected by treatment with tetracycline (especially doxycycline) [12, 32], though the treatment of C. trachomatis cervicitis includes concurrent treatment of sex partners to prevent re-infection [13].

Because C. trachomatis is an obligate parasite and infects the superficial lining epithelial cells for multiplication by binary fission, the periodontal lining epithelial cells were the target for specimen collection [62, 69]. The distinctive life cycle of chlamydia alternates between intracellular reticulate bodies that are contained within a membrane-bound vacuole of the host cell, and spore-like extracellular elementary bodies (EBs) [35]. Morphologically the EB is a small, spherical cell approximately 0.3 µm (300 nanometers) in diameter [35].

The primary objective of this study was to determine whether C. trachomatis could be detected in the periodontal lining epithelium of diseased periodontal sites using the cell collection methods and detection techniques that are commonly used to detect C. trachomatis in cervical specimens. The secondary objective of this study was to identify methodological issues when applying C. trachomatis detection techniques commonly used for cervical specimens to oral specimens.

### Materials and methods

The study design was cross-sectional to compare the presence of C. trachomatis in diseased and healthy periodontal sites in patients with established periodontitis and who also had three periodontally healthy teeth. “Established periodontitis” is defined as the presence of interproximal periodontal clinical attachment level ≥6 mm in two or more teeth and one or more interproximal periodontal sites with probed pocket depth ≥5 mm [46]. For 1 year (19 December 1994–15 December 1995), patients who presented for diagnosis and treatment planning appointments at the dental school clinic were screened for eligibility into the study. The inclusion criteria were: (1) 18–50 years of age at time of dental clinic visit, and (2) at least three teeth that met the case definition of established periodontitis [46], and (3) at least three teeth without periodontitis or gingivitis, i.e., healthy. Four additional criteria for exclusion from the study were applied to help control for possible confounding: (1) a history of specific antibiotics for treatment of C. trachomatis in the 3 months prior to the dental clinic visit, or (2) a history of periodontal curettage or surgery, or (3) a history of systemic disease characterized by neutrophil disorders (e.g., diabetes mellitus, Crohn’s disease, systemic lupus erythematosus, and ulcerative colitis), or (4) the inability to obtain informed consent.

Data were from the patient’s dental chart, an investigator administered questionnaire, and periodontal and mucosal cell specimens. Initial periodontal measurements were made by a dental student, confirmed by clinical faculty, and recorded in the patient chart. A single investigator (S.G.R.) screened all patients, confirmed periodontal measurements, performed informed consent, and collected all specimens.

The presence of C. trachomatis was assessed in cell specimens originating from three distinct locations in each patient’s mouth: (1) the lining epithelium of diseased periodontal sites, (2) the lining epithelium of healthy periodontal sites, and (3) a general collection of mucosal epithelium from the lining of the cheeks, floor of mouth, and tongue. Algorithms were used for the specific tooth selection. For the specimen from the diseased periodontal sites, cells were collected from the linings of the three periodontal sites with the most severe destruction measured (those used to diagnose established periodontitis) and pooled onto one microslide. Likewise, the specimen from the healthy periodontal sites was comprised of cells from the linings of the three periodontal sites with the smallest values of measured disease. The third microslide contained the pooled specimen of mucosal cells.

The periodontal measurements and cell collections were made using sterile periodontal probes (Michigan O) with millimeter demarcations and read to the lesser value. One probe was used for collecting from the pooled diseased sites and another from the pooled healthy sites. The periodontal probe was inserted to the base of the pocket, read, and then wiped along the lining epithelium in the area of the designated interproximal site. The probe was removed from the periodontal pocket and the cell specimen transferred onto the microslide using a rolling action of the probe on the microslide. The wiping action along the lining epithelium was repeated twice at each interproximal periodontal site for cell collection. The surface area of the lining epithelium sampled reflected approximately one-sixth of the tooth circumference.

The general mucosal cell collection was made using a cytobrush. Two strokes were made on each of the fol-