Etiology and Pathogenesis of Periodontal Disease

von
Alexandrina L Dumitrescu

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Etiology and Pathogenesis of Periodontal Disease – Dumitrescu

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There is a wide agreement on the etiological role of bacteria in human periodontal disease. Studies on the microbiota associated with periodontal disease have revealed a wide variety in the composition of the subgingival microflora (van Winkelhoff and de Graaff 1991).

The search for the etiological agents for destructive periodontal disease has been in progress for over 100 years. However, until recently, there were few consensus periodontal pathogens. Some of the reasons for the uncertainty in defining periodontal pathogens were determined by the following circumstances (Haffajee and Socransky 1994; Socransky et al. 1987):

1. **The complexity of the subgingival microbiota.** Over 300 species may be cultured from the periodontal pockets of different individuals, and 30–1,000 species may be recovered from a single site.

2. **Sample taking.** The physical constrains of a pocket make it difficult to obtain a representative sample from that pocket: that is, a sample that contains the pathogen and low number of contaminating species. If the sample is too large, the pathogen(s) would be diluted by noncontributory “contaminating” species. If the sample were too small or taken from the wrong place, one might miss the pathogen entirely.

3. **Difficulties in cultivation, characterization and identification of micro-organisms of plaque.** Many of the species in pockets are difficult or impossible to culture and difficult to identify. No single medium or environment is capable of recovering all of the organisms, which currently can be isolated from subgingival plaque. Many subgingival bacteria cannot be placed into recognized species. Some isolates are fastidious and are easily lost during characterization. Others are readily maintained but provide few positive results during routine characterization, and thus require special procedures for their identification.

4. **Mixed infections.** Not only single species are responsible for disease. If disease is caused by a combination of two or more microbial species, the complexity increases enormously. Mixed infections will not be readily discerned unless they attract attention by their repeated detection in extreme or problem cases.

5. **Opportunistic microbial species.** The opportunistic species may grow as a result of the disease, taking advantages of the conditions produced by the true pathogen. Changes in the environment such as the release of required substrates from damaged tissues or deepening of the periodontal pocket could be selected by certain opportunistic species. Their levels may increase concomitant with or after those of the true pathogens, and they may thus be difficult to distinguish experimentally.

6. **Disease activity.** Periodontal disease appears to progress with periods of exacerbation and remission. Ideally, a plaque sample should be taken at the peak of the disease activity. Failure to detect the peak of activity may lead to an underestimate of the contribution of a pathogen(s) to a given lesion.

7. **Multiple periodontal diseases in different subjects.** There appear to be multiple destructive periodontal diseases that, for the most part, cannot be differentiated on a clinical basis. Thus, disease types may be misclassified and inappropriately pooled.

8. **The possibility of multiple diseases in a subject.** Differences observed in clinical symptoms in different parts of the mouth may be explained by differences in levels of the pathogen or the stage of the
destructive process. Disease might have occurred in shallow lesions due to one species and in deepening lesions by a succession of other species. Disease occurring in one site in the mouth could be due to an agent that is different from the one inducing destruction at a second site at the same time.

9. The carrier state. Pathogens may be carried in low numbers in mouths that are free of destructive periodontal diseases (the so-called carrier state), making their role in disease more difficult to evaluate.

10. Virulent factors. Strains of putative pathogens may differ in virulence. A virulent clonal type might be detected in periodontally healthy subjects, whereas virulent clonal types might be present in subjects with periodontal disease. An inability to distinguish virulent from virulent clonal types would impede understanding.

11. Genetic virulence elements. It has been suggested that more virulent strains may harbor bacteriophages or plasmids. Bacterial plasmids are known to code for several virulence factors like invasiveness, adherence, and antimicrobial resistance as well as the production of toxins and noxious products. Several strains of _A. actinomycetemcomitans_ isolated from periodontal lesions of a rapidly destructive periodontitis patients have been described to have identical profiles consisting of four plasmids (Olsvik and Preus 1989). _A. actinomycetemcomitans_ phages were isolated from recently active periodontal sites in a patient suffering from prepubertal periodontitis, suggesting an association between periodontal breakdown and phage infection of _A. actinomycetemcomitans_ (Preus et al. 1987).

The task of defining the etiological agents of periodontal disease and subsequently the development of improved methods of classification, diagnosis, and treatment is clearly a cyclical process with continual revaluation and refinement (Socransky et al. 1987).

The criteria for defining pathogens in destructive periodontal diseases were initially based on Koch’s postulates. These postulates were: (1) the agent must be isolated from every case of the disease (2) it must not be recovered from cases of other forms of disease or non-pathogenically, and (3) after isolation and repeated growth in pure culture, the pathogen must induce disease in experimental animals. These postulates have been amended and extended in recent years, the criteria including association (the species should be found more frequently and in higher numbers in cases of an infection than in individuals without overt disease or with different forms of disease), elimination (elimination of a species should be accompanied by a parallel remission of disease), host response (if a species, or its antigens, gains access to underlying periodontal tissues and causes damage, it seems likely that the host will produce antibodies or a cellular immune response that is directed specifically at those species; thus, the host response could act as a pointer to the pathogens), virulence factors (potentially damaging metabolites produced, or properties possessed by certain species may be suggestive that that species could play a role in the disease process), animal studies (experimentally induced disease in dogs or monkeys, which can be manipulated to favor selection of single or subsets of species that may or may not induce pathology), and risk assessment (prospective studies are performed in which the risk of periodontal disease progression conferred by the presence of an organism at given levels may be assessed). The discrimination of a pathogen from a nonpathogenic species is not based on a single criterion but rather on a “weight of evidence” evaluation (Haffajee and Socransky 1994).

### 2.1 Virulence Factors of Periodontal Pathogens

The identification of pathogen(s) of an infectious disease, including periodontal diseases, leads inevitably to the question “how do these organisms cause the disease?” Thus, the study of potential virulence factors produced by oral species including periodontal pathogens is a very active area of research.

The term virulence is generally defined as the relative ability of an organism to cause disease or to interfere with a metabolic or physiological function of its host. The word derives from the Latin, “virulentus” or “full of poison.” Thus, virulence refers to the ability of a microbe to express pathogenicity (e.g., virulent), which is contrasted with nonpathogenic or avirulent organisms. Thus, virulence is not a separate property of the microbe, but is a complex interaction between the microbe and its host; this interaction being dependent upon many extrinsic factors of the environment. The characteristic endproducts of bacterial metabolism; the chemical composition of bacterial components; the ability of the intact bacterium or its parts to overwhelm host
2.2 Pathogens Suspected Currently in Destructive Periodontal Diseases

Several suspected pathogens have been identified to be involved in the destructive periodontal disease.

2.2.1 Aggregatibacter actinomycetemcomitans (Formerly Actinobacillus actinomycetemcomitans)

*A. actinomycetemcomitans* is small, nonmotile, gram-negative, saccharolytic, capnophilic, round-ended rod (Haffajee and Socransky 1994). During the last two decades, it has been shown that *Aggregatibacter actinomycetemcomitans* can be regarded as a major pathogen in destructive periodontal diseases (Slots et al. 1990a; van der Reijden et al. 2008; Slots and Ting 1999). The species is represented by six serotypes (a–f). Serotype b has been found more frequently and detected in higher numbers in active periodontitis lesions, whereas serotypes a and c have a stronger association with periodontal health (van der Reijden et al. 2008). Serotype b was significantly found more often in aggressive than in chronic periodontitis. They also found serotype b more frequently in periodontitis subjects under the age of 18 years (60.9%) in comparison to subjects older than 35 years (29%). The global distribution of the different *A. actinomycetemcomitans* serotypes is not homogeneous, which implies that the association between serotype and periodontal status may depend on the geographical location and/or ethinical status of the study population (van der Reijden et al. 2008; Fine et al. 2007).

2.2.1.1 Distribution

*Aggregatibacter actinomycetemcomitans* was first identified as a possible periodontal pathogen in 1975 in studies of localized juvenile periodontitis, now known as localized aggressive periodontitis (LAP) (Newman et al. 1976). Destructive periodontal disease in children is frequently associated with *A. actinomycetemcomitans*. Prepubertal periodontitis and other types of early onset periodontitis yield the organism in prevalence rates of 40–100% (Slots and Ting 1999). The close relationship between *A. actinomycetemcomitans* and early-onset periodontitis incriminates the organism in the development of many cases of the disease. *Localized juvenile periodontitis* is the most notorious disease associated with *A. actinomycetemcomitans*. Despite uncertainty about clinical diagnosis and prior periodontal therapy, studies have isolated *A. actinomycetemcomitans* from 75–100% of localized juvenile periodontitis lesions (Slots and Ting 1999).

*A. actinomycetemcomitans* is also associated with periodontitis lesions of Papillon-Lefèvre syndrome patients. Papillon-Lefèvre patients exhibit decreased function of monocytes, neutrophils and lymphocytes, which in part may be due to cytomegalovirus infection. It was hypothesized that the virally mediated host defense impairment may set the stage for overgrowth of subgingival *A. actinomycetemcomitans* (Slots and Ting 1999).
It was also showed that 30–40% and higher proportions of adult periodontitis patients exhibit the organism. In addition, the proportion of the subgingival microbiota comprising *A. actinomycetemcomitans* increases considerably with increasing periodontal probing depth. Also, *A. actinomycetemcomitans* has been detected four times as frequently in periodontal lesions with angular than with horizontal alveolar bone loss (Slots and Ting 1999). *A. actinomycetemcomitans* was also found to occur in periodontal sites undergoing active breakdown at levels 100-fold greater than those of the nonactive sites (Mandell 1984). Defining the “active” or “progressing” disease as a loss of connective tissue attachment of >2 mm during a 37-day monitoring period, Mandell et al. (1987) reported that 90% of the progressing sites (18/20) harbored *A. actinomycetemcomitans*, whereas only 44% of the stable or nonprogressing sites (7/16) harbored the organism (*P* < 0.05). Similar results were reported by Slots et al. (1986) who examined the occurrence of *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia* in 235 sites, including 104 from 61 untreated patients. Progressive lesions revealed a high prevalence of *A. actinomycetemcomitans* (50.0%), while nonprogressive sites demonstrated a significantly low prevalence of *A. actinomycetemcomitans* (4.8%). *A. actinomycetemcomitans* seems to be a particularly frequent organism in refractory periodontitis lesions, possibly due to the organism’s ability to invade gingival tissue and thereby evade the cleaning efforts of the dentist and the patient (Slots and Ting 1999).

*A. actinomycetemcomitans* can be found also in individuals with no history of destructive periodontal disease. Periodontally healthy children below 11 years of age exhibit an occurrence of *A. actinomycetemcomitans* from 0 to 26%. Adolescents with healthy periodontium or minimal disease exhibit less than 15% subgingival *A. actinomycetemcomitans* occurrence, while young adults with minimal periodontal disease reveal subgingival *A. actinomycetemcomitans* in a frequency of about 15%, although higher frequencies of occurrence have also been reported (Slots and Ting 1999).

In periodontitis patients, *A. actinomycetemcomitans* has been isolated not only from subgingival sites but also from extracrevicular locations in the mouth. Correlation analysis revealed significant positive association between the incidence of *A. actinomycetemcomitans* in infected individuals for deep and normal periodontal sites, periodontal pockets and cheek, tongue and saliva, and cheek and saliva (Muller et al. 1995).

In general, studies investigating successful and infected implants reveal differences in composition of the associated microbiota. Successful implants are reported to be populated with gram-positive coccioid cells, very few rods, a low ratio of anaerobe/aerobes and a low number of gram-negative anaerobes. *Infected and failing implants* show greater proportions of periodontal pathogens, including gram-negative anaerobe rods, motile rods, fusiform bacteria, and spirochetes, than nonfailing implants. These include large numbers of *Fusobacterium ssp.* and *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, *Peptostreptococcus micros*, *Campylobacter rectus*, *Capnocytophaga ssp.*, *P. intermedia*, and *P. gingivalis*. Other bacterial species such as *Pseudomonas aeruginosa*, *Enterobacteriaceae ssp.*, *Candida albicans*, *Staphylococcus epidermidis*, and *S. aureus* have also been identified around implants, but may reflect an opportunistic colonization of the plaque secondarily to antibiotic treatments (Norowski and Bumgardner 2009).

### 2.2.1.2 Virulence Factors

*A. actinomycetemcomitans* has been shown to possess a myriad of virulence factors that enhance its survival in the oral cavity and enable it to circumvent the host’s protective strategies (Fives-Taylor et al. 1999). Many of these virulence factors may be involved in the pathogenesis of periodontitis (Table 2.1). They include:

1. Factors that promote colonization and persistence in the oral cavity: Adhesins, Invasins, Bacteriocins, Antibiotic resistance.
2. Factors that interfere with the host’s defenses: Leukotoxin, Chemotactic inhibitors, Immunosuppressive proteins, Fc-binding proteins.
3. Factors that destroy host tissues: Cytotoxins, Collagenase, Bone resorption agents, Stimulators of inflammatory mediators.
4. Factors that inhibit host repair of tissues: Inhibitors of fibroblast proliferation, Inhibitors of bone formation.

**Adhesion of A. actinomycetemcomitans**

Most *A. actinomycetemcomitans* strains that have been tested adhere strongly to epithelial cells. Binding
Table 2.1 Virulence factors or antigens of *A. actinomycetemcomitans* studied that are associated with periodontal disease immunity (modified from Teng 2006) (with permission from Sage Publications)

<table>
<thead>
<tr>
<th>Virulence factor/antigen</th>
<th>Functional or immune characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF1 (14kDa)</td>
<td>Immuno-suppression of Th cells and down-regulate cytokine production</td>
</tr>
<tr>
<td>Cdt</td>
<td>Cell-cycle G2 arrest and apoptosis of human T-cells; stimulate pro-inflammatory cytokine production; CdtB-mediated nuclear transport in host cells; induce RANKL release in periodontal ligament cells</td>
</tr>
<tr>
<td>TadA and Flp-1</td>
<td>Bacterial colonization, nonspecific adherence, and fibrils assembly; immune IgG protection of alveolar bone loss in a rat oral challenge model</td>
</tr>
<tr>
<td>Leukotoxin</td>
<td>Lysis of monocytes/PMN, T-cells, NK cells, and HeLa cells (via β3-integrin and LFA-1) in a dose-dependent apoptosis or necrosis manner; stimulate human IgG response</td>
</tr>
<tr>
<td>CagE-homologue</td>
<td>Induce apoptosis of epithelia, endothelia, osteoblasts, and lymphocytes; activate; CD4+ T-cell-mediated immune response associated with osteoclastogenic activity</td>
</tr>
<tr>
<td>OMP-1</td>
<td>Induce IgG and CD4 + T-cell-mediated immunity and associated with osteoclastogenic activity</td>
</tr>
<tr>
<td>OPM-100</td>
<td>Bacterial adhesion, invasin, and serum resistance factor; induce host cytokine production</td>
</tr>
<tr>
<td>Surface proteins (SAM: 14–79kDa)</td>
<td>Stimulate protective immunity in s.c. mouse lesion model (via IgG activity)</td>
</tr>
<tr>
<td>GroEL (Hsp60)</td>
<td>Stimulate pro-inflammatory cytokine release; modulate antibacterial immunity; stimulate bone resorption in vitro (via osteoclast activity)</td>
</tr>
<tr>
<td>65-kDa protein</td>
<td>Modulate immune response by binding to IL-10R</td>
</tr>
<tr>
<td>LPS</td>
<td>Stimulate cytokine IL-1, TNF-α, IL-6, IL-8, and PGE2 release from host cells; induce bone osteoclastogenic activity</td>
</tr>
</tbody>
</table>

occurs very rapidly, reaching saturation levels within 1 h after infection (Fives-Taylor et al. 1999). Cell surface entities that mediate adherence include fimbriae, extracellular amorphous material and extracellular vesicles (Fives-Taylor et al. 1999). It was suggested that fimbriae most probably function in adherence of rough variants, whereas nonfimbrial components (such as vesicles) are probably involved in adherence of smooth, highly invasive strains (Meyer and Fives-Taylor 1994a, b). *A. actinomycetemcomitans* also produces poly-N-acetylglucosamine (PGA), a surface polysaccharide that mediates intercellular adhesion, biofilm formation and detergent resistance (Venketaraman et al. 2008).

In order to initiate disease in extraoral sites (such as endocarditis and osteomyelitis), *A. actinomycetemcomitans* must bind to the extracellular matrix, the complex network of proteins and polysaccharides that is secreted by, and underlies epithelial and endothelial cells and surrounds connective tissue. The major component of the extracellular matrix is collagen (Fives-Taylor et al. 1999). Mintz and Fives-Taylor (1999) showed that multiple strains of *A. actinomycetemcomitans* bind to several types of connective tissue collagen and fibronectin, but not to the plasma protein, fibrinogen. Binding, therefore is highly specific. All the collagen types were demonstrated to be substrates for the binding of *A. actinomycetemcomitans* strains. A degree of specificity in the binding of *A. actinomycetemcomitans* SUNY465 to various collagen molecules was demonstrated by the almost complete lack of binding to type IV collagen, which is only found in basement membranes (Mintz and Fives-Taylor 1999). Outer membrane proteins on the bacterial cell surface are essential for binding. The binding of *A. actinomycetemcomitans* to the insoluble form of proteins that are major structural components of the extracellular matrix must aid the organism in its spread and colonization, not only in oral sites but at extraoral sites as well (Fives-Taylor et al. 1999).

**Antibiotic Resistance**

Roe et al. (1995) examined 18 clinical isolates of *A. actinomycetemcomitans* from 16 patients with periodontitis. Eighty-two percent of the *A. actinomycetemcomitans* isolates were resistant to tetracyclines, and frequently employed antibiotic used as an adjunct to mechanical debridement in the treatment of localized juvenile periodontitis, and carried the Tet B resistance determinant. It was also that the TetB determinant transferred at frequencies of 3.5 × 10⁻⁵–2.5 × 10⁻⁴ per *A. actinomycetemcomitans* recipient and 1 × 10⁻⁸–6 × 10⁻⁹ per *H. influenzae* recipient. Marked reduction of subgingival *A. actinomycetemcomitans* associated with the resolution of clinical signs of localized juvenile periodontitis after 7 days course with a combination of systemic metronidazole and amoxicillin was reported (Christersson et al. 1989).
Bone Resorption

A characteristic feature of periodontal disease is the loss of bone supporting the teeth. *A. actinomycetemcomitans* has been shown to stimulate bone resorption by several different mechanisms: lipopolysaccharide, proteolysis-sensitive factor in microvesicles and surface-associated material (Fives-Taylor et al. 1999; Wilson et al. 1985). Surface-associated material has recently been identified as the molecular chaperone, GroEL. The chaperone appears to act in a direct way with the major bone-resorbing cell population, the osteoclast (Meghji et al. 1992, 1994; Fives-Taylor et al. 1999).

Collagenase

As previously stated, collagen is the most abundant constituent of the extracellular matrix. A major feature of periodontal disease is a marked reduction in gingival collagen fiber density. Collagenase activity is associated with *A. actinomycetemcomitans* (Fives-Taylor et al. 1999).

Cytotoxins

One of the most important cell types within the gingival connective tissue is the fibroblast. Fibroblasts are a major source of collagen and confer structural integrity to the tissue. Many oral bacteria express toxins that inhibit human fibroblast proliferation, but the heat-labile cytotoxin produced by *A. actinomycetemcomitans* is especially cytotoxic. The toxin is considered a virulence factor due to its impact on fibroblast viability (Fives-Taylor et al. 1999).

Extracellular Membranous Vesicles

Almost all strains of *A. actinomycetemcomitans* examined extrude membrane vesicles from their surface. The vesicles associated with SUNY 465 grown on agar are fibrillar membranous extensions with knob-like ends. These vesicles often contain leukotoxin, endotoxin, bone resorption activity and a bacteriocin. *A. actinomycetemcomitans* vesicles must also contain adhesins, since their addition to a weak adherent or nonadherent strains significantly increases the ability of those strains to attach to epithelial cells (Fives-Taylor et al. 1999).

*Bacteriocins* are proteins produced by bacteria that are lethal for other strains and species of bacteria. These toxic agents can confer a colonization advantage for the bacterium by lessening the ecological pressures associated with competition by other organisms for both nutrients and space (Fives-Taylor et al. 1999). Hammond et al. (1987) has showed that it enhances its chance to colonisation by producing in vivo an extracellular factor, actinobacillin, that is directly toxic to two major plaque formers that primarily colonize the tooth surface, *S.sanguinis* and *Actinomyces viscosus*. It was also showed that bacteriocin produces alterations in the cell permeability of target bacteria, with resultant leakage of RNA, DNA, and other essential intracellular macromolecules and cofactors (Fives-Taylor et al. 1999).

Leukotoxin

One of the best studied *A. actinomycetemcomitans* virulence factors is leukotoxin, a 114-kDa secreted lipoprotein that belongs to the RTX family of pore-forming bacterial toxins. *A. actinomycetemcomitans* leukotoxin has been shown to kill polymorphonuclear leukocytes (PMNs) and macrophages isolated specifically from humans and Old World primates. Human subjects harboring highly leukotoxic strains of *A. actinomycetemcomitans* are more likely to develop periodontitis than subjects harboring minimally leukotoxic strains. These findings suggest that leukotoxin may play a role in host cell killing and immune evasion in vivo (Kolodrubetz et al. 1989; Venketaraman et al. 2008; Balashova et al. 2006; Diaz et al. 2006).

Fc-Binding Proteins

Bacterial immunoglobulin-binding proteins, or Fc receptors, are proteins that bind to the Fc portion of Igs. These receptors are postulated to interfere with complement- or antibody-dependent host immune mechanisms, as well as certain immune functions. If other proteins compete for binding to this region of PMNs, binding of the antibody may be inhibited, and
2.2 Pathogens Suspected Currently in Destructive Periodontal Diseases

thereby inhibit phagocytosis (Fives-Taylor et al. 1999). Mintz and Fives-Taylor (1994) demonstrated the presence of Ig Fc receptors on the surface cells of several *A. actinomyctemcomitans* strains. The murine monoclonal antibodies of unrelated specificity were of the IgG subclass of Igs. It was proposed that Fc receptors may be another factor that aids in the persistence of *A. actinomyctemcomitans* at extracellular sites during the disease process. Tolo and Helgeland (1991) showed that release of Fc-binding components from bacteria may interfere with the phagocytic activity (a 90% phagocytosis reduction was noted), complement function and down-regulation of B-cell proliferation in the periodontal infiltrates.

**Lipopolysaccharide**

Lipopolysaccharides (endotoxins) have a high potential for causing destruction of an array of host cells and tissues. Tissue destruction is a key feature of periodontal diseases; thus, the lipopolysaccharide of *A. actinomycetemcomitans* has been extensively characterized (Low concentrations of *A. actinomycetemcomitans* lipopolysaccharide stimulate macrophages to produce interleukins (interleukin-1α, interleukin-1β) and tumor necrosis factor (TNF), cytokines involved in tissue inflammation and bone resorption. These data suggest that macrophages that migrate to gingival sites of *A. actinomycetemcomitans* infection will be stimulated to produce these cytokines, which may then be involved in gingival inflammation and alveolar bone resorption (Fives-Taylor et al. 1999; Rogers et al. 2007).

**Immunosuppressive Factors**

Host defense mechanisms play a major role in controlling concentrations of bacterial communities in dental plaque. *A. actinomycetemcomitans* has been shown to elaborate many factors capable of suppressing these host defense mechanisms (Fives-Taylor et al. 1999). It was showed that *A. actinomycetemcomitans* also produces a 60-kDa protein, which down regulates both T- and B-cell responsiveness through the activation of a subpopulation of B lymphocytes (Shenker et al. 1990). Now, this factor is known as cytolethal distending toxin (CDT), which induces apoptosis to lymphocytes (Ohara et al. 2004). It was also found that the bacteria produce a novel 14-kDa substance (designated suppressive factor 1), which suppresses cell proliferation and cytokine production by mouse splenic T cells (Kurita-Ochiai and Ochiai 1996). SF1 is a 14kDa protein, which inhibits T-cell proliferation and production of Th1 (IL-2 and IFN) and Th2 (IL-4 and IL-5) cytokines by ConA-stimulated splenic T cells. Therefore, this molecule could affect the induction of humoral and/or cell-mediated immune responses through the modulation of the T-cell responses, including cytokine production (Kurita-Ochiai and Ochiai 1996). A distinct decrease in the helper-to-suppressor T-cell ratio of patients with either the juvenile or the rapidly progressive forms of early-onset periodontal disease was showed by Kinane et al. (1989). Separate analysis of patients with either the juvenile or rapidly progressive types of early-onset periodontal disease showed both to have reduced CD4+/CD8+ ratios (means 0.91 and 0.92) relative to their controls (means 1.57 and 1.45), which were both statistically significant (*P* < 0.05) (Fig. 2.1).

**Inhibitors of Polymorphonuclear Leukocyte Function**

The host’s first line of defense against invading bacteria is the recruitment of phagocytes to the area. The ability to disrupt chemotaxis permits the invading organism to survive this major challenge from the host. *A. actinomycetemcomitans* secretes a low-molecular-weight compound that inhibits polymorphonuclear leukocyte (PMN) chemotaxis. The inhibitory activity is abrogated by treatment with proteinase K, suggesting that the compound is proteinaceous in nature. Another activity of PMNs is the killing of bacteria by a wide variety of potent antibacterial agents that are gained when the PMNs fuse with lysosomes. *A. actinomycetemcomitans* has been shown to be capable of inhibiting PMNs from producing some of these compounds, and it is intrinsically resistant to others. A heat-stable protein in *A. actinomycetemcomitans* inhibits the production of hydrogen peroxide by PMNs, and many strains are naturally resistant to high concentrations of hydrogen peroxide. Furthermore, *A. actinomycetemcomitans* has been shown to be resistant to several of the cationic peptides, known as defensins, which are found in neutrophils (Fives-Taylor et al. 1999).
Penetration of Epithelial Cells

It was showed that *A. actinomycetemcomitans* has the ability to penetrate the gingival epithelium. An in vitro model of infection of the human KB cell line was developed to investigate the interaction of *A. actinomycetemcomitans* with epithelial cells. The results showed that the degree of invasion by *A. actinomycetemcomitans* was greater in KB cells than in cells originating from nonoral sources. TEM analysis revealed that the *A. actinomycetemcomitans* occurred singly in vacuoles, and it was suggested that colonial morphology may be involved in determining the invasive capability of this organism (Meyer et al. 1991). Saglie et al. (1986) reported that Gram-stained sections from diseased sites (advanced and localized juvenile periodontitis) contained large numbers of bacteria in the oral epithelium and adjacent connective tissue. 87% of the sections showed more than 20 bacteria in the oral epithelium and 6% of the sections showed between 10 and 20 bacteria.

Immunofluorescence examination of frozen gingival section from patients with localized juvenile periodontitis with pooled antisera to *A. actinomycetemcomitans* revealed staining in subepithelial tissues, intracellularly and extracellularly in 80% of the lesions. Staining for *A. actinomycetemcomitans* antigens was detected extracellularly, in the connective tissue, in 69% of the biopsies. This finding indicates penetration by, or transfer of, bacteria or bacterial antigens into the connective tissue (Christersson et al. 1987a). The numbers of *A. actinomycetemcomitans* cultivable from pockets infected with this bacterium correlated with tissue infiltration by this microorganism when assessed by both immunofluorescence microscopy (*P* = 0.013) and by culture from the minced gingival biopsy (*P* = 0.002) (Christersson et al. 1987b).

2.2.1.3 *A. actinomycetemcomitans* and Systemic Diseases

It was recently revealed that organisms of the HACEK group (*Haemophilus* spp., *A. actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*), and *A. actinomycetemcomitans*, in particular, are associated with systemic diseases distant from the oral cavity (Fine et al. 2006). Nonoral manifestations of periodontal infections merit attention, especially in light of the recent discovery of putative relationships between periodontal disease and coronary heart disease, preterm birth and cerebral infarction. Most likely, systemically healthy individuals are at low risk of becoming ill from dental focal infections. In
immunocompromised patients, the oral cavity may constitute a significant reservoir for serious pathogens. Periodontal disease, odontogenic abscesses and endodontic infections increase the likelihood of dissemination of oral microorganisms to nonoral sites. As *A. actinomycetemcomitans* is an organism that avidly attaches to both soft and hard tissues of the tooth, and positions itself adjacent to the permeable junctional and pocket epithelium, it should come as no surprise that *A. actinomycetemcomitans* has been isolated from a number of organs distant from the oral cavity and is capable of causing serious infections in humans. Such infections include fascial plane infection, heart infection, endocarditis, pericarditis, lung infection, necrotizing pneumonia, mediastinitis, mediastinal abscess, transdiaphragmatic infection, endophthalmitis, skin infection, vertebral osteomyelitis, cervical lymphadenitis, submandibular space abscess, and urinary tract infection (Fine et al. 2006 Kaplan et al. 1989).

### 2.2.2 Porphyromonas gingivalis

*Porphyromonas* (*P.*) *gingivalis* is the second intensively studied probable periodontal pathogen. Isolates of this species are gram-negative, anaerobic, nonmotile asaccharolytic rods that usually exhibit coccal to short rod morphologies. *P. gingivalis* is a member of the much investigated black-pigmented Bacteroides group (Haffajee and Socransky 1994).

#### 2.2.2.1 Prevalence in Periodontal Disease

Most authors agree that *periodontally healthy* children and adolescents harbor few or no *P. gingivalis* in the subgingival microbiota. *P. gingivalis* has been described in 37–63% of *localized juvenile periodontitis* patients; however, the organism is rarely found at the debut of the disease and tends to comprise only a small part of the microbiota in early disease stages. In contrast, *P. gingivalis* is a predominant organism in *generalized juvenile periodontitis* and may assume pathogenetic significance in the disease. Adults having a healthy and minimally diseased periodontium reveal subgingival *P. gingivalis* in less than 10% of study sites. On the other hand, 40–100% of *adult periodontitis* patients may yield the organism. Furthermore, *P. gingivalis* comprises a considerably higher proportion of the subgingival microbiota in *deep than in shallow periodontal pockets* (Slots and Ting 1999; Ready et al. 2008), and in *progressive deep periodontal lesions* than in nonprogressive sites (31.6 vs. 7.4%) (Slots et al. 1986) *Infected and failing implants* show greater proportions of periodontal pathogens, including gram-negative anaerobe rods, motile rods, fusiform bacteria, and spirochetes, than nonfailing implants. These include large numbers of *Fusobacterium ssp.* and *Prevotella intermedia*, *A. actinomycetemcomitans*, *Peptostreptococcus micros*, *Campylobacter rectus*, *Capnocytophaga spp.*, *P. intermedia*, and *P. gingivalis* (Norowski and Bumgardner 2009).

Several studies suggested that the outcome of *periodontal treatment* is better if particular suspected pathogens, notably *P. gingivalis* and *A. actinomycetemcomitans*, can no longer be detected after therapy (Slots and Rosling 1983; Christersson et al. 1985; Kornman and Robertson 1985; Haffajee et al. 1988a, b; Rodenburg et al. 1990), and that positive sites are at greater risk for further break down (Slots et al. 1986, Bragd et al. 1987; Slots and Listgarten 1988; Fine,1994; Rams et al. 1996; Brochut et al. 2005). However, despite the fact that non-surgical, mechanical periodontal treatment as well as self-performed plaque control is effective in reducing the numbers of *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans* at periodontal sites, these organisms re-establish themselves rapidly in most subjects once adult periodontitis is present, indicating that effective plaque control is constantly required (Doungudomdacha et al. 2001; Johnson et al. 2008).

#### 2.2.2.2 Virulence Factors

**Lipopolysaccharide**

Identical to Gram-negative prokaryotes, *P. gingivalis* synthesizes a lipopolysaccharide. The cell envelope of the gram-negative bacterium consists of two distinct membranes: the inner (cytoplasmic) membrane; and the outer membrane. The outer membrane of gram-negative bacteria lies external to the peptidoglycan and is attached to it by selected lipoproteins. These lipoproteins or murein lipoproteins attach by both covalent and noncovalent bonds to protein units within *P. gingivalis* and to the outer membrane by their lipid moieties.
The outer membrane of gram-negative bacteria is asymmetric, the outer leaflet of which contains the lipopolysaccharide. The lipopolysaccharide is a very large molecule, with estimates ranging from 10 kDa and larger. Its amphipathic character is a result of one end of the molecule, the hydrophilic end consisting of the polysaccharide or O-specific (somatic) antigen, which is exposed to the environment on the exterior surface of the outer membrane, and the core region, buried within the outer leaflet, which connects the O-antigen to the hydrophobic end of the molecule or lipid A. This complex lipid is embedded in the lipid portion of the outer membrane leaflet (Holt et al. 1999). Chemical dissection of the lipopolysaccharide into its component parts (O-antigen, core, lipid A) has permitted the determination of the biologically active components of the parent molecule (Yoshimura et al. 2009) (Fig. 2.2) (Table 2.2).

LPS does function as a significant cytotoxin as well as inducer of several host derived cyto- and chemokines. The lipopolysaccharide of P. gingivalis is chemically different from that found in the well studied and benchmark enteric lipopolysaccharide. These chemical and structural differences more than likely reflect the functional differences between the two molecules and may relate to their role in the pathogenesis of periodontal disease. The low biological activity of P. gingivalis, especially its very low endotoxicity, may reflect the organisms’ ability to colonize and grow in sterile tissue undetected by the host (Holt et al. 1999).

**Adhesion and Coaggregation**

As part of the repertoire of P. gingivalis virulence factors, it has been shown to possess distinct molecules/structures that are essential to interactions with the host. Specifically, this species has been shown to be capable of adhering to a variety of host tissues and cells, and to invade these cells and multiply (Holt and Ebersole 2005).

Coaggregation is a phenomenon that describes the specific interaction of pairs of oral bacteria via cognate binding. Many species of oral bacteria have been shown to demonstrate this function, presumably related to the development of the complex biofilms of the oral cavity. Thus, intergeneric coaggregation clearly contributes to the characteristics of the complex microbial ecology of biofilms established in the multiple habitats of the oral cavity. P. gingivalis is capable of coaggregating with Actinomyces naeslundii two (Actinomyces viscosus), Streptococcus gordonii, S. mitis and filmbriated Streptococcus salivarius. This interaction is altered by heat treatment, various sugars, amino acids, cation chelation, and protease treatment, suggesting a specific ligand–receptor interaction (Holt and Ebersole 2005).

The initial event in the pathogenicity of P. gingivalis is its interaction (adherence) in the oral cavity. To accomplish this, P. gingivalis employs several bacterial components: fimbriae, proteases, hemagglutinins, and lipopolysaccharide (Holt and Ebersole 2005). Fimbriae or pili are proteinaceous, filamentous
appendages that protrude outwards from the bacterial cell surface and play a crucial role in virulence by stimulating bacterial attachment to host cells or tissues (Holt and Ebersole 2005).

The first fimbriae are called major, long, or FimA fimbriae, and the second ones are referred to as minor, short, or Mfa1 fimbriae (Yoshimura et al. 2009). Major fimbriae are filamentous components on the cell surface, and their subunit protein, fimbillin (FimA) reportedly acts on bacterial interactions with host tissues by mediating bacterial adhesion and colonization in targeted sites. Major fimbriae are capable of binding specifically to and activating various host cells such as human epithelial cells, endothelial cells, spleen cells, and peripheral blood monocytes, resulting in the release of cytokines including interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor-α (TNF-α), as well as cell adhesion molecules including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and P- and E-selectins. In addition, P. gingivalis major fimbriae have been shown necessary for bacterial invasion to host cells (Amano et al. 2004).

Minor fimbriae were shown to be short fimbria like appendages in an fimA (major fimbria-deficient) mutant of strain ATCC 33277. A subunit protein of a minor fimbiae (Mfa1) encoding the mfa1 gene was shown to be different in size (67 kDa in contrast to 41 kDa of major fimbria subunit) and antigenicity from that of major fimbriae. Although a fimA mutant revealed a significant reduction of adhesive potential to saliva-coated

### Table 2.2 Virulence factors or antigens of P. gingivalis that are associated with periodontal immunity (modified from Teng 2006) (with permission from sage publications)

<table>
<thead>
<tr>
<th>Virulence factor/antigen</th>
<th>Functional or immune characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>dpp</td>
<td>Abscess formation and lethality</td>
</tr>
<tr>
<td>HagA (hemagglutinin)</td>
<td>Tissue/cell invasion</td>
</tr>
<tr>
<td>HagB (hemagglutinin)</td>
<td>Stimulate strong IgG and Th immune responses; induce immune protection</td>
</tr>
<tr>
<td>Gingipains (RgpA, RgpB, Kgp)</td>
<td>Tissue destruction and alter cytokine/chemokine and IgGs bio-activity (i.e., IL-12, TNFα, C3 and C5, IgG/A)</td>
</tr>
<tr>
<td></td>
<td>Stimulate protease-activated receptors (PAR-1 and -4) or platelet-activating factor associated with Th1 and IgG2 immune responses</td>
</tr>
<tr>
<td>Gingipain-R1 (Rgp-A and -B) (RgpA is critical for protection)</td>
<td>Stimulate immune protection in a murine oral challenge and s.c. abscess model, respectively</td>
</tr>
<tr>
<td>Cysteine proteinases (Arg- and Lys-)</td>
<td>Induce RANKL production; disrupt PMN function</td>
</tr>
<tr>
<td>Rgp-Kgp/adhesin-based peptide complex, or Kgp-DNA vaccine</td>
<td>Induce protection in mouse s.c. abscess model or rat oral challenge model (via IgG activity); protection via IgG activity; immune protection related to IgG4/Th2 response; mAb to RgpA inhibit P.g. colonization in the experimental human subjects</td>
</tr>
<tr>
<td>Hemoglobin-binding domain</td>
<td>Stimulate immune protection in a rat model (via both IgG and Th2/Th1-ratio-driven responses)</td>
</tr>
<tr>
<td>Fimbriae (FimA, etc.)</td>
<td>Bacterial colonization, induce host IgA, IgG, and Th1 immune responses; stimulate pro-inflammatory cytokine release; stimulate CD14, TLR2 and 4, CD11a/CD18</td>
</tr>
<tr>
<td></td>
<td>Endothelial atherosclerotic change; induce periodontal bone loss in rats and mice (Immunization induces protective IgG/A immunity in a guinea pig subcutaneous lesion model and a germ-free rat model, and T-cell epitope mapped.)</td>
</tr>
<tr>
<td></td>
<td>A 20-mer P8-peptide (T- and B-cell epitope) induces immune protection in a mouse s.c. lesion model</td>
</tr>
<tr>
<td>LPS</td>
<td>Escape immune recognition; innate hypo-responsiveness</td>
</tr>
<tr>
<td></td>
<td>Activate APC’s immuno-suppressive effects (i.e., increase ILT-3 and B7-H1 release); change CD14 and LTR expressions</td>
</tr>
<tr>
<td>ClpP</td>
<td>Modulate various cytokine expressions</td>
</tr>
<tr>
<td>GroEL (Hsp60)</td>
<td>Bacterial invasion into epithelium</td>
</tr>
<tr>
<td>LPS: Lipid-A</td>
<td>Stimulate NF-κ and host cytokine production</td>
</tr>
<tr>
<td>Outer membrane proteins (PG32 and PG33, or OMP40/41)</td>
<td>Stimulate immune protection in a mouse s.c. abscess model (via IgG activity); stimulate PBMC T-cells to produce IL-17</td>
</tr>
<tr>
<td>Capsular PS</td>
<td>Stimulate immune protection in mouse oral challenge model (via IgG activity)</td>
</tr>
<tr>
<td>Ag53 (53 kDa)</td>
<td>Stimulate strong IgG2 and Th1 immune responses; both B-cell epitopes and dominant T-cell epitope (hu-HLA-DRB1 restricted) mapped</td>
</tr>
<tr>
<td>ClpP</td>
<td></td>
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<tr>
<td>GroEL (Hsp60)</td>
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<tr>
<td>LPS: Lipid-A</td>
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hydroxyapatite, gingival epithelial cells, and fibroblasts, as well as bone adsorption capability, in an orally infected rat model, minor fimbriae purified from *P. gingivalis* ATCC 33277 markedly induced IL-1α, IL-β, IL-6, and TNF-α cytokine expression in mouse peritoneal macrophages (Amano et al. 2004).

### Proteinases

One of the potential virulence proprieties displayed by *P. gingivalis* is the high proteolytic activity associated with these organisms. The primary function of proteases and peptidases secreted by asaccharolytic bacteria such as *P. gingivalis* is, most likely, to provide nutrients for growth. At least eight secreted proteinases have now been described for *P. gingivalis* and their concerted activities, in addition to providing amino acids, peptides and hemin for growth, including processing of essential cell surface components and provision of substrates for bacterial cell adhesion. The proteinases are also involved directly in tissue invasion and destruction by bacteria, and in evasion and modulation of host immune defenses. Specific examples of tissue degradation and attenuation of host defense mechanisms include: the degradation of extracellular matrix proteins; activation of matrix metalloproteinases (MMPs); inactivation of plasma proteinase inhibitors; cleavage of cell-surface receptors; activation or inactivation of complement factors and cytokines; activation of the kallikrein-kinin cascade; stimulation of apoptotic cell death; and disruption of PMN functions (Holt et al. 1999; Holt and Ebersole 2005). The adhesive and enzymatic functions of *P. gingivalis* proteinases are intricately interconnected. *P. gingivalis* cells bind to and degrade human plasma fibronectin, laminin, fibrinogen and collagen. The adhesion and degradation processes involve the activities of fimbriae and of the Arg-X-specific and Lys-X-specific cysteine proteinases. Hydrolysis of fibronectin or other matrix proteins such as collagen by the *P. gingivalis* Arg-X proteinases RgpA and RgpB enhances the binding of fimbriae to these substrates. The proteinases are able to expose sequences within host matrix protein molecules that carry C-terminal Arg residues, thus promoting adhesion of the organism through a fimbrial–arginine interaction. This may represent one mechanism by which initial gingivitis progresses to more severe periodontitis. Increased proteolytic activity associated with infection of the gingival sulcus could expose previously hidden receptors that would then enhance colonization by *P. gingivalis* (Lamont and Jenkinson 2000).

The best studied are the cysteine proteinases, or the “gingipains,” with specificities for cleavage after arginine and lysine residues (Curtis et al. 2001; Holt et al. 1999; Travis et al. 1997). Gingipains, originally considered “trypsin-like proteinases,” actually comprise a group of cysteine endopeptidases that have been reported to account for at least 85% of the general proteolytic activity displayed by *P. gingivalis*, and 100% of the expressed “trypsin-like activity.” The gingipains, both soluble and cell-associated, are the products of three genes, *rgpA*, *rgpB*, and *kgp*, encoding these cysteine proteinases. The product of the *kgp* gene (Lys-gingipain, gingipain K) cleaves polypeptide chains exclusively after lysine residues. The products of the *rgpA* and *rgpB* genes (Arggingipain, gingipain R) are proteinases specific for arginine residues (Holt and Ebersole 2005).

Gingipains contribute to the virulence potential of *P. gingivalis* in a multifactorial way, especially by influencing the binding of the bacterium to host tissues. These proteinases may play a role in binding to host cells, either by binding to a cognate receptor or by exposing cryptoplopeptide chains exclusively after lysine residues. The mature forms of Arg- gingipain A and Lys- gingipain possess a catalytic domain and three or four hemagglutinin/adhesin (HA) domains (HA1 to HA4) linked by strong noncovalent bonds. The HA domains of Arg- gingipain A and Lys- gingipain share a high degree of homology (over 97%) and have been implicated in the adherence of *P. gingivalis* to gingival epithelial cells. Gingipains have been shown to play important physiological roles, more particularly in controlling the expression of virulence factors and the stability and/or processing of extracellular and cell-surface proteins (Andrian et al. 2006).

Other proteases of *P. gingivalis* are less well studied than the gingipains with regard to their participation in infection by this microorganism and the inflammatory events of periodontitis. However, several of them might play some role in the host destructive events. Genes coding for collagenase, a protease-hemagglutinin gene, a broadspectrum protease, an endothelin converting like enzyme, a dipeptidyl peptidase, and a reported protease called periodontain have all been isolated and
2.2 Pathogens Suspected Currently in Destructive Periodontal Diseases

Invasion of Oral Epithelial Cells

*P. gingivalis* has developed adaptive strategies to invade gingival epithelial cells and overcome the protective defense mechanisms of epithelial cells. *P. gingivalis* adheres to and invades epithelial cells by targeting specific host receptors, modulating host signaling events and deregulating the host cytokine network. Interactions between *P. gingivalis* and epithelial cells lead to the activation of several complex signaling cascades, which ultimately regulate the transcription of target genes that encode effectors and regulators of the immune response. Effectors of the innate immune system, proinflammatory cytokines, chemokines, MMPs, and antimicrobial peptides are up-regulated and may have a direct impact on disease progression and the inflammation processes, which may contribute to bacterial persistence and the progression of chronic manifestations of periodontal diseases (Andrian et al. 2006; Yilmaz 2008).

2.2.3 *Tannerella forsythia* (Formerly *Bacteroides forsythus*)

The second member of the red complex of Socransky et al. (1998) is *T. forsythia*. The original isolate, identified as a “fusiform Bacteroides,” was first reported in the literature by Tanner et al. in 1979. *T. forsythia* is described as a gram-negative anaerobic fusiform isolated from the human oral cavity. Because of its unique growth requirements (hemin, menadione, L-cysteine, and *N*-acetylneuraminic acid) and the fact that it is a somewhat difficult to grow, its precise role in the severe bone and tissue destruction at sites from which it can be isolated, remains to be determined (Tanner and Izard 2006).

2.2.3.1 Distribution

The microorganism was frequently isolated along with *P. gingivalis* from cases of active chronic periodontitis, and has been frequently associated with severe periodontal disease compared to healthy controls. Periodontitis that progresses posttherapy, “refractory” periodontitis, represents a particularly aggressive form of disease, which has also been associated with detection of *T. forsythia*. Moreover, *T. forsythia* was frequently associated with *P. gingivalis* colonization and was elevated in older groups of patients. Detection of *T. forsythia* was associated with early periodontitis in a comparison of subgingival and tongue samples from healthy subjects and those with early periodontitis. Detection associated with bleeding on probing (BOP) or attachment loss in adolescents has also been recorded, further suggesting an association with early periodontitis, which reveals a relationship to existing disease and potentially active disease sites (Holt and Ebersole 2005).

Risk factors for periodontitis have also been linked with increased detection of *T. forsythia*. Detection of *T. forsythia* was associated with subjects who were smokers, positive for aspartate aminotransferase activity, or interleukin-1 genotype (PST test). Systemic disease is frequently associated with lowered resistance to infection, including periodontal infections. *T. forsythia* was associated with viral diseases, subjects infected with Human immunodeficiency virus (HIV), diabetes, and Papillon–Lefèvre syndrome (Tanner and Izard 2006).

2.2.3.2 Virulence Factors

*T. forsythia* expresses a robust enzymatic repertoire related to its asaccharolytic physiology. *T. forsythia* produces an enzymatic peptidase activity that degrades benzoyl-DL arginine-naphthylamide (BANA), the activity of which appears related to sites of periodontal tissue destruction, and that was originally described as a trypsin-like protease. *T. forsythia* produces lipoproteins (BfLP) that were shown to activate gingival fibroblasts to produce elevated levels of interleukin-6 and TNF-α. Interleukin-6 is known to function in the induction of several acute phase proteins in liver cells involved in cytotoxic T-cell differentiation, as well as the growth of myeloma/plasmacytoma cells. It is also capable of inducing B-cells. Interleukin-6 also induces bone resorption by osteoclast formation with soluble interleukin-6 receptor. In addition, as described above, it is known that *T. forsythia* synthesizes a sialidase and a trypsin-like enzyme, which are thought to be involved in host-cell virulence (Holt and Ebersole 2005).

Moreover, it was demonstrated that *T. forsythia* had the ability to induce apoptosis. Functionally, this activity
of *T. forsythia* might be considered part of the progression of periodontitis. *T. forsythia* appears to invade the periodontal pocket along with *P. gingivalis* (and *T. denticola*), and these species could be attacked by the host’s white blood cells. The apoptotic-inducing activity could result in the elimination of host immune or preimmune cells; loss of these host immune cells from the developing periodontal pocket would support bacterial colonization of the pocket and the potential rapid progression of the disease (Holt and Ebersole 2005).

### 2.2.4 Treponema denticola

*T. denticola* is but one member of the oral treponemes. These rapidly motile, obligatory anaerobic gram-negative bacteria have been estimated to account for approximately 50% of the total bacteria present in a periodontal lesion. *T. denticola* increases to large numbers in adult periodontitis but is almost undetectable in oral health (Holt and Ebersole 2005; Sakamoto et al. 2005). Treponemes, as a group, often localize at the forefront of periodontal infections, in chronic periodontitis and necrotizing ulcerative gingivitis (NUG), in both invasive disease and when confined to the pocket (Ellen and Galimanas 2005).

Socransky and coworkers’ studies have emphasized the cohabitation of oral bacterial species and have demonstrated a pattern of bacterial flora succession, emphasizing a relationship of the various clusters with periodontal disease progression and severity. The cluster composed of highly proteolytic species *P. gingivalis*, *T. forsythia*, and *T. denticola*, the so-called “red” cluster, has the strongest relationship to advanced and progressive periodontitis (Ellen and Galimanas 2005).

Tissue invasion is a hallmark of spirochetal infections, and many oral treponemes have the capacity to penetrate the gingiva as individual species in experimental models or as cohabitants of natural mixed infections. The known virulence factors that determine their invasiveness are the various proteins involved in the synthesis and energetics of flagellar motility, chemotaxis proteins, and the chymotrypsin-like protease, dentilisin (Ellen and Galimanas 2005). *T. denticola* possesses several peptidases associated with its outer sheath. One of these, a prolyl-phenylalanine specific protease, also called chymotrypsin-like protease, appears to be important in *T. denticola* virulence. Isolated in its native form, it appears to exist as a complex protein with a molecular mass of approximately 100 kDa. When denatured, the 100kDa native protein was separated into three peptides, with molecular masses of 72, 43, and 38 kDa. The 72 kDa protein has been named dentilisin (Holt and Ebersole 2005).

Periodontal pathogens in the proteolytic complex have the ability to degrade human matrix components, which would enhance their tissue penetration and those of neighboring species. *T. denticola* binds extracellular matrix proteins and proteoglycans, elaborates an enzyme that degrades hyaluronic acid and chondroitin sulfate, while its subtilisin family serine protease, dentilisin, has a wide range of protein substrates including fibronectin, laminin, and fibrinogen (Ellen and Galimanas 2005).

*T. denticola* also synthesizes two low-iron induced outer membrane proteins, HbpA and HbpB that bind hemin. These proteins appear to be necessary for efficient iron utilization, although this microorganism has the ability to replace the function of these proteins by accessing a variety of sources of host iron for nutrition (Ellen and Galimanas 2005).

Elevated levels of *T. denticola* were identified along with nearly a dozen other species in sulfide-positive compared with sulfide-negative sites. The results suggested that the sulfide levels in the pockets reflected the proportion of bacteria, whose metabolism resulted in the production of sulfide as an end-product (Ellen and Galimanas 2005).

### 2.2.5 Fusobacterium nucleatum

*Fusobacterium nucleatum*, which refers to a group of three subspecies (nucleatum, vincentii, and polymorphum), is a gram-negative anaerobic bacterium associated with gingivitis and chronic periodontitis. This periodontopathogen has also been implicated in a variety of nonoral infections such as pleuropulmonary infections, urinary tract infections, endocarditis, and intra-amniotic infections (Grenier and Grignon 2006; Boldstad et al. 1996).

The ability of *F. nucleatum* to coaggregate with many plaque bacteria suggests that it acts as a *microbial bridge*
between early and late colonisers. In addition to its ability to coaggregate with many oral bacteria, *F. nucleatum* has also been described as an important initiator organism by promoting physico-chemical changes in the gingival sulcus, allowing pathogenic successors to establish and proliferate. An important change associated with the onset of periodontal disease is the increased alkalisation of the gingival sulcus. Ammonia produced by the metabolism of amino acids found in gingival crevicular fluid and released by the breakdown of host tissues, leads to an increase in pH above 8.0, thereby promoting the proliferation of acid-sensitive pathogenic bacteria. It was also reported that *F. nucleatum* alters its gene expression according to environmental pH. The ability to form a biofilm and coaggregate could be an important virulence mechanism, and may explain the finding in a study on alkalini-resistant bacteria in root canal systems that *F. nucleatum* is capable of surviving at pH 9.0 (Zilm and Rogers 2007). It was recently also suggested that *F. nucleatum* facilitates invasion of host cells by *P. ginvivalis* (Saito et al. 2008).

Apart from its metabolic versatility, its cell-surface properties enable it to attach to epithelial cells, collagen, gingival epithelial cells and other bacterial genera, but not with other *Fusobacteria*. However, recently it was reported that *F. nucleatum* has been shown to coaggregate and form a biofilm, which may be important in the organism’s persistence during the transition from health to disease in vivo (Zilm and Rogers 2007).

*F. nucleatum* was demonstrated to be a significant marker for destructive periodontal disease in adult subjects (van Winkelhoff et al. 2002; Papapanou et al. 2002; Mosca et al. 2007), was identified more often in active sites than in inactive sites (Dzink et al. 1988), and was associated with higher pocket sulfide levels in chronic periodontitis subjects (Torresyap et al. 2003). It has demonstrated high IL-1 and TGF-β production by gingival mononuclear cells extracted from adult periodontitis tissues after stimulation with the putative periodontopathic bacteria, *F. nucleatum* (Gemell and Seymour 1993).

### 2.2.6 *Prevotella intermedia*

*P. intermedia*, a black-pigmented gram-negative obligate anaerobic nonmotile rod, has received a considerable interest as it was reported to be an important periodontal pathogen, and it was significantly prevalent in patients with chronic periodontitis, aggressive periodontitis, destructive periodontitis, puberty-associated gingivitis and acute NUG (Socransky et al. 1998; Dahlén et al. 1990; Dzink et al. 1983; Moore et al. 1985). In an Australian population, Hamlet et al. (2001) revealed that the odds of a site being *P. intermedia* positive were marginally greater (1.16) for pockets deeper than 3 mm. When Kook et al. (2005) performed microbial screening for predicting the outcome of periodontal treatment in Koreans using a polymerase chain reaction, a close association was revealed between the presence of BOP and the presence of *Prevotella intermedia*. Furthermore, the sites harboring both *T. forsythia* and *P. intermedia* at the baseline had a poorer response to treatment than the sites where these two species were not detected.

Additionally, in vitro invasion of *Prevotella intermedia* to human gingival epithelial cells has been observed (Dorn et al. 1998), and intracellular division of *Prevotella intermedia* in cultured human gingival fibroblasts has been observed by Dogan et al. (2000). *Prevotella intermedia* induced proinflammatory cytokine expression in human gingival epithelial cells (Sugiyama et al. 2002) and human periodontal ligament (hPDL) cells (Yamamoto et al. 2006; Guan et al. 2006). Pelt et al. (2002) also demonstrated that *P. intermedia* induced pro-MMP-2 and pro-MMP-9 expression in fetal mouse osteoblasts.

In *P. intermedia*, several proteases have been described, among them being trypsin-like serine proteases, a dipeptidyl peptidase IV, CPs (Shibata et al. 2002; Guan et al. 2006; Deschner et al. 2003) and a new cysteine protease from the cysteine-histidine-dyad class, interpain A (Mallorquí-Fernández et al. 2008). *Prevotella intermedia* also possess various types of fimbriae (surface appendages). Some of these surface structures mediate the adherence of the organism to several mammalian erythrocytes, resulting in the agglutination of the erythrocytes (Leung et al. 1999).

### 2.2.7 *Campylobacter rectus*

*Campylobacter rectus* was previously called *Wolinella recta*; it was renamed by Vandamme et al. (1991). *Campylobacter rectus*, a gram-negative, microaerophilic,
round ended, straight, nonglycolytic and motile bacterium has been proposed to play a pathogenic role in human periodontitis. *Campylobacter rectus* has often been detected in large numbers in deeper subgingival pockets (Dzink et al. 1985; Grenier and Mayrand 1996; Tanner et al. 1981; Gmürr and Guggenheim 1994; Lai et al. 1992; Listgarten et al. 1993; Lopez et al. 1995; van Steenbergen et al. 1993c; Ibara et al. 2003; Moore et al. 1983; Rams et al. 1993 Macuch and Tanner 2000), and has been implicated in adult periodontitis, rapidly advancing periodontitis and periodontitis associated with certain conditions (pregnancy) (Yokoyama et al. 2005, 2008) and diseases such as AIDS and diabetes (Zambon et al. 1988, 1990). It has been reported that, in adult periodontitis, these organisms were detected more frequently than *P. gingivalis* or *A. actinomycescomitans* by using PCR methods, and correlate with clinical parameters, including probing depth and BOP (Ibara et al. 2003). Furthermore, *C. rectus* is also found in combination with other suspected periodontopathogens (Socransky et al. 1998; Haffajee et al. 1988b). Longitudinal studies suggest that *C. rectus* is one of the major species that characterizes sites converting from health to disease (Tanner et al. 1998), and its levels are reduced after periodontal treatment (Haffajee et al. 1988a; Bostanci et al. 2007).

Surface components such as the flagellum, surface layer (S-layer), and GroEL-like protein (GroEL) have been reported as possible virulence factors of the microorganism, and can induce the expression of various inflammatory mediators by host cells (Wang et al. 1998; Ishihara et al. 2001; Hinode et al. 1998, 2002; Braun et al. 1999; Miyamoto et al. 1998). More specifically, *C. rectus* lipopolysaccharide stimulates the production of PGE2, interleukin-1β (IL-1β), and IL-6 by gingival fibroblasts, whereas the crystalline surface layer stimulates the secretion of IL-6, IL-8, and TNF-α by HEp-2 cells derived from a human pharyngeal cancer. *C. rectus* GroEL, which is a 64-kDa heat-shock protein, also stimulates the production of IL-6 and IL-8 by human gingival cells (Yokoyama et al. 2008).

### 2.2.8 Eikenella corrodens

*E. corrodens* is a gram-negative, facultative anaerobe, capnophilic, saccharolytic, regular, small rod with blut ends, and may also cause extra-oral infections including abscesses, endocarditis, arthritis, osteomyelitis, keratitis, conjunctivitis and cellulitis (Haffajee and Socransky 1994; Chen and Wilson 1992; Fujise et al. 2004; Chang and Huang 2005; Karunakaran et al. 2004).

*E. corrodens* is found predominantly in subgingival plaque in patients with advanced periodontitis (Nommenmacher et al. 2001; Noiri et al. 2001; Salari and Kadkhoda 2004). The frequency of cultivable *E. corrodens* from subgingival sulci of healthy, adult periodontitis and juvenile periodontitis patients was reported as 10, 52 and 59%, by Chen et al. (1989). In periodontitis patients, *E. corrodens* was related to disease active sites compared to inactive sites either before or after successful periodontal therapy, emerging as possible periodontal pathogen (Tanner et al. 1987). Additionally, the mono-infection of germ-free rats with *E. corrodens* causes periodontal disease with severe alveolar bone loss (Crawford et al. 1977; Noiri et al. 2001; Cortelli and Cortelli 2003; Apolônio et al. 2007).

It was reported that *E. corrodens* 1,073 has a cell-associated N-acetyl-D-galactosamine (GalNAc) specific lectin-like substance (EcLS) that mediates its adherence to various host tissue cell surfaces and oral bacteria, induces ICAM-1 production by human oral epithelial cells, and also stimulates the proliferation of murine B cells. EcLS is a large molecule and is composed of several components including 25-, 45- and 300-kDa proteins. Moreover, it has been shown that soluble products from *E. corrodens* 1,073 induce the secretion and the expression of IL-8 by a human oral epidermoid carcinoma cell line (KB) (Yamada et al. 2002). Nevertheless, despite the many virulence factors exhibited by *E. corrodens*, such as lipopolysaccharides, proteins of the outer membrane, adhesins and the exopolysaccharide layer (Chen and Wilson 1992), antagonistic substances produced by this bacterium have only recently been reported (Apolônio et al. 2007, 2008).

### 2.2.9 Parvimonas micra (Previously Peptostreptococcus micros or Micromonas micros)

One of the suspected pathogens related to periodontal disease is the gram-positive anaerobic coccus *Peptostreptococcus micros*. Although it is considered a natural commensal of the oral cavity, elevated levels of
2.2 Pathogens Suspected Currently in Destructive Periodontal Diseases

this organism are not only associated with chronic, aggressive periodontitis and with active sites of periodontal destruction (Rams et al. 1992a; von Troili-Larsen et al. 1995; Socransky et al. 1998; Choi et al. 2000; Gajardo et al. 2005; van Winkelhoff et al. 2005; Salari and Kadkhoda 2004; Haffajee et al. 2004; Lee et al. 2003; van Winkelhoff et al. 2002), but also with periodontal decline in old adults (Swoboda et al. 2008). Nonnenmacher et al. (2001) evaluated the prevalence of Porphyromonas gingivalis (now known as localized aggressive) periodontitis. Significantly higher numbers of P. micros were present in smokers and associated with moderate and deep pockets (van der Velden et al. 2003; Gomes et al. 2006). When heavy smokers were considered, higher counts of total bacteria, M. micros, and D. pneumosintes were observed (Gomes et al. 2006). P. micros has also been associated with infected dental root canals (Gomes et al. 2004) and dentoalveolar infection (Dymock et al. 1996; Kuriyama et al. 2007).

Van Dalen et al. (1993) reported the existence of two morphotypes (rough and smooth) of P. micros, which differ in the presence of cell-associated fibril-like appendages, in the composition of cell wall proteins, the surface hydrophobicity, and in the ability to lyse erythrocytes. Both morphotypes may be recovered from subgingival plaque and are likely acting as opportunistic pathogens, in association with gram-negative bacteria, to contribute to periodontitis (van Dalen et al. 1998; Grenier and Bouclin 2006).

The virulence factors produced by P. micros, which may play a role in the pathogenesis of periodontitis, are poorly characterized. P. micros is able to adhere to epithelial cells and to other periodontopathogens, including Porphyromonas gingivalis and Fusobacterium nucleatum (Kremer et al. 1999; Kremer and van Steenbergen 2000). P. micros cells have also the ability to bind A. actinomycetemcomitans lipopolysaccharide on their surface, thus significantly increasing their capacity to induce TNF-α production by human macrophages (Yoshioka et al. 2005). It was also showed that the P. micros cell wall preparation induced intracellular signaling pathways, leading to an increased production of proinflammatory cytokines, chemokines and MMP-9 by macrophages (Tanabe et al. 2007). Recently, Grenier and Bouclin (2006) provided evidence that the proteolytic and plasmin-acquired activities of P. micros may facilitate the dissemination of bacterial cells through a reconstructed basement membrane. Kremer et al. (1999) reported the ability of P. micros, more particularly the smooth morphotype, to adhere to oral epithelial cells. Gelatinase and hyaluronidase activities produced by P. micros have also been reported (Ng et al. 1998; Tam and Chan 1984).

2.2.10 Selenomonas species

Selenomonas sp. are gram-negative, curved, saccharolytic rods that may be recognized by their curved shape, tumbling motility, and in good preparations, by the presence of a tuft of flagella inserted in the concave site. The organisms have been somewhat difficult to grow and speciate (Haffajee and Socransky 1994).

In patients with generalized aggressive periodontitis, S. sputigena was the most frequently detected bacterial species, often at high levels of about 20% of the total bacterial population. This gram-negative, multilflagellated, motile, anaerobic rod has also been previously associated with necrotizing ulcerative periodontitis (Gmürr et al. 2004), rapidly progressive periodontitis (Kamma et al. 1995), and in smokers with early onset periodontitis (Kamma and Nakou 1997) and active periodontitis lesions (Haffajee et al. 1984; Tanner et al. 1998; Faveri et al. 2008). Other predominant Selenomonas sp. are Selenomonas sp. oral clone EW084, Selenomonas sp. oral clone EW076, Selenomonas sp. oral clone FT050, Selenomonas sp. strain GAA14, Selenomonas sp. Oral clone P2PA_80, and Selenomonas noxia. All of these have been previously associated with oral infections (Kumar et al. 2005; Paster et al. 2001, 2002; Faveri et al. 2008). Selenomonas noxia was found at significantly higher levels in periodontal pocket sulfide levels (Torresyap et al. 2003).

2.2.11 Eubacterium species

Eubacterium nodatum, Eubacterium brachy, and Eubacterium timidum are gram-positive, strictly anaerobic, small and somewhat pleomorphic rods. They are often difficult to cultivate, particularly on primary
isolation, and appear to grow better in roll tubes than on blood agar plates. The *Eubacterium* sp. appear to be promising candidates as periodontal pathogens; however, difficulty in their cultivation has slowed assessment of their contribution (Haffajee and Socransky 1994).

Moore and Moore (1994) used the roll tube cultural technique to examine the proportions of bacterial species in subgingival plaque samples from subjects with various forms of periodontitis and gingivitis, and in healthy subjects. They found that *E. nodatum* was absent or in low proportions in periodontal health and various forms of gingivitis, but was present in higher proportions in moderate periodontitis (2%), generalized early onset periodontitis (8%), localized juvenile periodontitis (6%), early onset periodontitis (5%) and adult periodontitis (2%). *E. nodatum* was in the top two to 14 species enumerated in these different periodontal states (Haffajee et al. 2006). More recent studies have confirmed an association of *E. nodatum* with periodontitis using molecular techniques. Using species-specific oligonucleotide probes, Booth et al. (2004), revealed that simple comparisons of *E. nodatum* at shallow sites in periodontitis patients and healthy controls and at deep and shallow sites in the patient group suggested that *E. nodatum* was associated with periodontal disease. A strong association of *E. nodatum* and *T. denti-cola* with periodontitis, whether in the presence or absence of high levels of the consensus pathogens, was recently emphasized (Haffajee et al. 2006). Similar data were reported by Papapanou et al. (2000) and Colombo et al. (2002). *E. nodatum* was found to be significantly higher in current smokers than nonsmokers (Haffajee and Socransky 2001, 2006).

### 2.2.12 Streptococcus intermedius

Cultural studies of the last decade have suggested the possibility that some of the *streptococcal* sp. are associated with and may contribute to periodontal disease progression. At this time, evidence suggests that *S. intermedius* or closely related species may contribute to disease progression in subsets of periodontal patients (Haffajee and Socransky 1994).

Polymorphisms in the cluster of IL-1 genes have been significantly associated with the severity of adult periodontitis. When microbiological parameters in IL-1 genotype negative and positive adult subjects with a range of periodontitis severities were compared, it was observed that the proportion of IL-1 genotype positive subjects that exhibited mean counts of specific subgingival species above selected thresholds was significantly higher than the proportion of genotype negative subjects. Significantly higher mean counts of *S. intermedius* were detected at periodontal pockets >6 mm in subjects who were genotype positive when compared with genotype negative subjects. The increase was due to increased numbers of cells of these species rather than a major shift in proportion (Socransky et al. 2000).

When the mean frequency of detection of *Streptococcus intermedius* in epithelial cell samples from 49 periodontitis subjects was evaluated, the obtained percent was 36% (Colombo et al. 2006).

When subgingival microflora in periodontal disease patients was assessed, the detection rates for *A. actinomycetemcomitans, E. corrodens, S. noxia* and *S. intermedius* varied between 58–83% in smokers and 55–82% in nonsmokers. *S. intermedius* was the only species for which the detection rates in smokers and nonsmokers differed more than 10%-U (Boström et al. 2001).

### 2.2.13 Other Species

Interest has grown in groups of species not commonly found in the subgingival plaque as initiators or possibly contributors to the pathogenesis of periodontal disease, particularly in individuals who have responded poorly to periodontal therapy. Species not commonly thought to be present in subgingival plaque can be found in a proportion of such subjects or even in subjects who have not received periodontal treatment (Haffajee and Socransky 1994).

In order to elucidate the range of species of nonoral, gram-negative, facultatively anaerobic rods in human periodontitis, Slots et al. (1990a, b) has studied 3,050 advanced periodontitis patients and obtained pooled samples from 9,150 deep periodontal pockets. *Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Klebsiella oxytoca* and *Enterobacter agglomerans* were the most frequently isolated species, accounting for more than 50% of all strains. It was suggested that some species of this group of organisms can be cofactors in destructive periodontitis and they should not be given the benefit of doubt in the treatment. These bacteria are often recovered from the subgingival microbiota of patients considered to be clinically refractory to mechanical and antibiotic periodontal treatment.
2.3 Ecologic Relationships Among Bacterial Species and Between Bacterial Species and the Host

Preliminary clinical findings indicate that systemic ciprofloxacin administration, but not conventional periodontal therapy, may cure periodontal infections with these organisms (Slots et al. 1990c).

Enterococci are bile-tolerant, facultatively anaerobic, chaining gram-positive cocci that are common inhabitants of the human gastrointestinal and genitourinary tracts as normal commensals. They can cause a variety of diseases in humans, infecting the urinary tract, bloodstream, endocardium, abdomen, biliary tract, burn wounds, and indwelling foreign devices (Jett et al. 1994). Enterococci are also able to colonize a variety of other sites, including the oral cavity, where they have been associated with oral mucosal lesions in immunocompromised patients, periodontitis and root canal infections (Pinheiro et al. 2006; Wahlin and Holm 1988; Rams et al. 1992b). Of the enterococcal sp. associated with colonization and infection in humans, Enterococcus faecalis is the most common (Pinheiro et al. 2006).

However, very few studies have evaluated the correlation between the prevalence of E. faecalis and periodontal diseases (Rams et al. 1992b; Souto et al. 2006; Gonçalves Lde et al. 2007; Pinheiro et al. 2006). Rams et al. (1992b) detected E. faecalis in 1% of early onset periodontitis and 5.1% of chronic periodontitis patients using cultural methods, whereas Souto and Colombo (2008) found a much higher prevalence of this species (80%) in a large number of subgingival biofilm samples from periodontitis patients. In addition, these authors observed that this bacterium was much more prevalent in healthy sites from periodontitis patients as compared to sites in periodontally healthy individuals. Souto and Colombo (2008) showed a significantly higher frequency of E. faecalis in saliva (40.5%) and subgingival biofilm samples (47.8%) from periodontitis patients compared to periodontally healthy controls (14.6 and 17.1%, respectively). Similar data were reported by Colombo et al. (2002), who examined the presence and levels of E. faecalis in the subgingival microbiota of untreated periodontitis patients and healthy controls using the checkerboard method (Souto and Colombo 2008).

Staphylococcal sp., in particular Staphylococcus epidermidis and S. aureus, dominate the microbial aetiology of prosthetic valve endocarditis. However, the oral cavity has been established as a source of the organisms for native valve endocarditis (NVE), where Viridans streptococci are responsible for 50% of cases (Debelian et al. 1994).

Staphylococci have been isolated from the oral cavity, but they are not considered resident oral bacteria and are generally regarded as transient organisms. While it is not clear whether there is a causal relationship between staphylococci and chronic periodontal disease (Dahlén and Wikström 1995), staphylococci have been isolated from subgingival sites within periodontitis patients (Rams et al. 1990a, b; Slots et al. 1990a, b; Dahlén and Wikström 1995; Murdoch et al. 2004). However, few subgingival plaque samples have been collected from nondiseased sites, and consequently, it has not been possible to determine if the isolation of staphylococci was because of the diseased state of the tissues or whether staphylococci are a feature of all subgingival sites (Murdoch et al. 2004). In a recent study, staphylococci were isolated from 54% diseased subgingival and 43% healthy subgingival sites in over 50% periodontitis patients and from 29% healthy subgingival sites in 54% controls. No significant differences in the frequency of isolation or numbers of staphylococci isolated from diseased and healthy sites were noted. Staphylococcus epidermidis was the predominant oral species.

Periodontitis is caused by mixed bacterial infection in the oral cavity. Pathogenic subgingival microorganisms may be responsible for initation/progression of periodontal diseases. Among them include Porphyromonas gingivalis, Tannerella forsythia, Aggregatibacter (Actinobacillus) actinomycetemcomitans, Prevotella intermedia, and Treponema denticola. These bacteria are usually found in combination in periodontal pockets rather than alone, suggesting that some of the bacteria may cause destruction of the periodontal tissue in a cooperative manner (Yoneda et al. 2005; Haffajee and Socransky 1994).

T. forsythia has been found in subgingival plaques in patients with severe periodontitis along with P. gingivalis (Jervoe-Storm et al. 2005; Tanner and Izard 2006; Simonson et al. 1992), and cooperates with P. gingivalis to form severe abscesses in rabbits and mice (Yoneda et al. 2001). It was suggested that P. gingivalis with either T. forsythia or T. denticola directly induces synergistic IL-6 protein production and that gingipains play a role in this synergistic effect (Tamai et al. 2009). Sonicated cell extracts from T. forsythia
stimulate the growth of *P. gingivalis* (Yoneda et al. 2005), while the outer membrane vesicles produced by *P. gingivalis* enhance the attachment to and invasion of epithelial cells by *T. forsythia* (Inagaki et al. 2006). It was also observed that *P. gingivalis, T. denticola*, and *T. forsythia* stimulate the secretion of proinflammatory cytokines (IL-1β, IL-6), chemokines (IL-8, RANTES), PGE₂, and MMP-9 in a macrophage/epithelial cell coculture model. This indicates that these periodontal pathogens have a strong potential for activating host-mediated destructive processes. No synergistic effects on cytokine, chemokine, PGE₂, or MMP-9 production were observed for the bacterial mixtures compared to monoinfections by individual bacterial species. This study supports the view that bacterial species of the red complex act in concert to increase the levels of proinflammatory mediators and MMP-9 in periodontal tissues, a phenomenon that may significantly contribute to the progression of periodontitis (Bodet et al. 2006).

Moreover, in a murine abscess model, combinations of *P. gingivalis–Fusobacterium nucleatum, P. gingivalis–T. denticola*, and *P. gingivalis–A. actinomycetemcomitans* exhibited enhanced virulence compared to monoinfections (Ebersole et al. 1997; Chen et al. 1996; Kesavalu et al. 1998; Yoneda et al. 2005).

The data from these investigations suggests that different complexes of microbial species reside in different periodontal pockets. The major ecological determinants are probably provided by the interactions that take place between the host and resident subgingival species, and between the different species. These interactions can produce a series of selective pressure, which determine the composition of the microbiota in the subgingival sites (Socransky et al. 1988).

Several prerequisites are necessary for periodontal disease initiation and progression: the virulent periodontal pathogen, the local environment and the host susceptibility.

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**The virulent periodontal pathogen**

It seems unlikely, however, that a single factor alone will be entirely responsible for the virulence of *P. gingivalis*. Several studies have shown, by the use of animal models, that virulent and avirulent strains exist within the species of *P. gingivalis*; differences in hydrophobicity and hemagglutinating activity between pigmented and non-pigmented strains, however, suggest additionally that structural differences also occurred between both groups of strains (Shah et al. 1989; Neiders et al. 1989; Smalley et al. 1989). Besides the virulence strain, another requirement is that the organism possesses all of the necessary genetic elements. Some of these elements might be missing in a strain inhabiting the gingival crevice area but could be received from other strains of that species via bacteriophages, plasmids or transposons. Thus, periodontally healthy sites might be colonized with periodontal pathogens without a full complement of genes needed to lead to tissue destruction (Haffajee and Socransky 1994).

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**The local environment**

In a complex ecosystem, such as the periodontal pocket, antagonistic and commensal relationships are to be expected. Most of these would have minimal impact on the health of the periodontium. However, such relationships may be causally related to the maintenance of health or the initiation and progression of disease, particularly when putative periodontal pathogens are involved. Certain viridans streptococci, by virtue of their ability to produce hydrogen peroxide, appear to promote periodontal health by keeping the numbers of potentially pathogenic organisms below the threshold level necessary to initiate disease. Certain types of periodontal disease may therefore result from an ecological imbalance, which arises from the following sequence of events: first, unknown factors promote the relative outgrowth of an organism such as *A. actinomycetemcomitans*, which produces a factor inhibitory to the growth of certain streptococcal sp.; this result in a reduction of local hydrogen-peroxide production, which in turn permits the outgrowth of various periodontal pathogens (Hillman et al. 1985). The local subgingival environment can affect disease pathogenesis in other ways. It has been showed that iron is an essential requirement for the growth of most microorganisms. Pathogenic microorganisms have developed specific mechanisms to obtain iron from host protein. The most extensively studied iron uptake system is that used by many aerobic organisms and depends on the production of siderophores. Iron restriction in the environment increases the expression of a number of virulence factors of *P. gingivalis* (Barua et al. 1990).

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**The host susceptibility**

A number of host factors have been suggested to affect the initiation and rate of progression of periodontal diseases. Such factors include defects in PMN
levels or function, a poorly regulated immune response, smoking, diet and various systemic diseases. In HIV-positive and diabetic subjects, it has been shown that the periodontal lesions, for the most part, appeared to be related to already suspected periodontal pathogens and not to some novel species. It seems that altered host susceptibility may change the rate of disease progression in affected individuals, and largely, the periodontal pathogens are likely to be the same as those found in uncompromised subjects (Haffajee and Socransky 1994).

2.4 The Possible Role of Viruses in the Pathogenesis of Periodontal Diseases

Even though specific infectious agents are of key importance in the development of periodontitis, it is unlikely that a single agent or even a small group of pathogens are the sole cause or modulator of this heterogeneous disease (Slots 2005).

2.4.1 HIV Infection

HIV is a retrovirus with special affinity for the CD4 receptor molecule, which is situated on the surface of T-helper lymphocytes. Other infected cell populations include monocytes and macrophages, Langerhans’ cells, B lymphocytes, endothelial cells and cells in the brain. As a result of the HIV infection, the number of CD4+ cells decreases. The ratio of T-helper to T-suppressor lymphocytes (the CD4+:CD8+ cell ratio) is increasingly reduced with the progression of disease, and the function of the entire immune system of the host is widely affected. The host thereby becomes susceptible to several infectious diseases and neoplasms (Holmstrup and Westergaards 1998).

A consensus has been reached by the EC-WHO on the classification of the oral manifestations of HIV infection and their diagnostic criteria, based on presumptive and definitive criteria. The former relate to the initial clinical appearance of the lesion and the latter are often the result of special investigations. Candidiasis, hairy leukoplakia, specific forms of periodontal disease [linear gingival erythema (LGE), necrotising-(ulcerative) gingivitis (NG) and necrotising (ulcerative) periodontitis], Kaposi’s sarcoma and non-Hodgkin’s lymphoma are strongly associated with HIV infection. Lesions less commonly associated with HIV infection includes: bacterial infections, melanotic hyperpigmentation, necrotising (ulcerative) stomatitis, salivary gland disease, thrombocytopenic purpura, ulcerations, viral infections, while lesions seen in HIV infection, but not indicative of the disease, are: bacterial infections, cat-scratch disease, drug reactions, epitheloid (bacillary) angiomatosis, fungal infection other than candidiasis, neurologic disturbances, recurrent aphthous stomatitis, and viral infections (Table 2.3).

• Linear gingival erythema

LGE is a distinct fiery red band along the margin of the gingiva. The amount of erythema is disproportionately intense for the amount of plaque seen. No ulceration is present and, according to the EC-WHO criteria, there is

<table>
<thead>
<tr>
<th>Lesions strongly associated with HIV infection</th>
<th>Lesions less commonly associated with HIV infection includes</th>
<th>Lesions seen in HIV infection, but not indicative of the disease</th>
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<tbody>
<tr>
<td>Candidiasis (erythematous, pseudomembranous)</td>
<td>Bacterial infections (Mycobacterium avium-intracellulare, Mycobacterium tuberculosis)</td>
<td>Bacterial infections (Actinomyces israelii, Escherichia coli, Klebsiella pneumoniae)</td>
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<tr>
<td>Hairy leukoplakia</td>
<td>Melanotic hyperpigmentation</td>
<td>Cat-scratch disease</td>
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<tr>
<td>Specific forms of periodontal disease [linear gingival erythema, necrotising-(ulcerative) gingivitis and necrotising (ulcerative) periodontitis], Kaposi’s sarcoma, Non-Hodgkin’s lymphoma</td>
<td>Necrotising (ulcerative) stomatitis</td>
<td>Drug reactions</td>
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<td></td>
<td>Salivary gland disease</td>
<td>Epitheloid (bacillary) angiomatosis</td>
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<td>Thrombocytopenic purpura</td>
<td>Fungal infection other than candidiasis</td>
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<td>Ulcersations NOS</td>
<td>Neurologic disturbances</td>
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<td>Viral infections (Herpes simplex virus (HSV), Human papillomavirus, Varicella-zoster virus)</td>
<td>Recurrent aphthous stomatitis</td>
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<tr>
<td></td>
<td></td>
<td>Viral infections (Cytomegalovirus, Molluscum contagiosum)</td>
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</table>
no evidence of pocketing or attachment loss. A characteristic feature of this type of lesion is that it does not respond well to improved oral hygiene nor to scaling (Holmstrup and Westergaards 1998). Treatment includes debridement by a dental professional, twice-daily rinses with a 0.12% chlorhexidine gluconate suspension for 2 weeks, and improved home oral hygiene (Reznik 2005).

The prevalence of LGE in HIV-infected population varies from 0 to 49%, and this considerable variation can be due to the lack of clear diagnostic standardization and the heterogeneity of the populations studies (Cappuyns et al. 2005).

- **Necrotising-(ulcerative) gingivitis**

Necrotising gingivitis, necrotising periodontitis, and necrotizing stomatitis may be different stages of the same disease (Cappuyns et al. 2005). HIV-related necrotizing gingivitis is defined by ECWHO as destruction of one or more interdental papillae. In the acute stage of the process ulceration, necrosis and sloughing may be seen with ready hemorrhage and characteristic fetor.

The available information about the microbiology of HIV-associated necrotizing gingivitis is limited. The isolated organisms include *Borrelia*, gram-positive cocci, beta-hemolytic streptococci and *C.albicans* (Holmstrup and Westergaards 1998).

- **Necrotizing (ulcerative) periodontitis (NP)**

Although necrotizing gingivitis and necrotizing periodontitis may reflect the same disease entity, they are differentiated by the rapid destruction of soft tissue in the former condition and hard tissue in the latter. Necrotizing ulcerative periodontitis is a marker of severe immune suppression. The condition is characterized by severe pain, loosening of teeth, bleeding, fetid odor, ulcerated gingival papillae, and rapid loss of bone and soft tissue. Patients often refer to the pain as “deep jaw pain” (Holmstrup and Westergaards 1998; Tirwomwe et al. 2007).

The occurrence of *P.gingivalis*, spirochetes and motile eubacteria in periodontitis has been found to be similar in HIV-infected patients and systemically healthy adults. Moreover, the microflora found in HIV-associated periodontitis was similar to that of classical adult periodontitis, except that *P.gingivalis* was more prevalent in conventional periodontitis (Holmstrup and Westergaards 1998; Feller and Lemmer 2008).

It has been suggested that NP may be used as a marker for immune deterioration, with a 95% predictive value that CD4+ cell counts have decreased below 200 cells μl⁻¹). If untreated, the cumulative probability of death within 24 months is 72.9% (Cappuyns et al. 2005). NG/NP, in otherwise systemically healthy individuals, is strongly correlated with HIV infection, with a predictive value of 69.6%. It is recommended that patients presenting with these conditions be encouraged to undergo testing to establish their HIV status for appropriate referrals and management (Shangase et al. 2004).

Necrotizing periodontitis in HIV-infected patients does not always respond to conventional treatment with scaling and improved oral hygiene (Holmstrup and Westergaards 1998). However, treatment includes removal of dental plaque, calculus, and necrotic soft tissues, utilizing a 0.12% chlorhexidine gluconate or 10% povidone-iodine lavage, and institution of antibiotic therapy (Reznik 2005).

- **Necrotizing stomatitis**

Necrotizing stomatitis is described as a localized acute, painful ulceronectric lesion of the oral mucosa that exposes underlying bone or penetrates or extends into contiguous tissues. The lesions may extend from areas of necrotizing periodontitis. The lesions are acute, extensively destructive, rapidly progressive, ulcerative, and necrotizing. Usually, the lesions extend from the gingiva into adjacent mucosa and bone causing massive destruction of the oral soft tissues and underlying bone. Like HIV-associated periodontitis, it appears to be related to the immune depletion caused by HIV infection and, importantly, it may be life-threatening. Extensive denudation of bone may result in sequestration. Progression of necrotizing periodontitis to necrotizing stomatitis may subsequently result in progressive osseous destruction with the development of oroantral fistula and osteitis (Holmstrup and Westergaards 1998).

- **Conventional chronic and aggressive periodontitis**

In addition to the specific forms of periodontal disease described below, it should be appreciated that chronic marginal gingivitis and adult periodontitis can occur in patients with HIV infection. The clinical appearances of these conditions may, however, be altered or exaggerated as a result of immunosuppression (WHO-EC 1993).

The reported prevalence rates of periodontitis among HIV-seropositive patients show considerable variation (5–69%) (Holmstrup and Westergaards 1998). Recently, Kroidl et al. (2005) revealed that compared with data of oral diseases of the pre-HAART (highly active antiretroviral therapy) era, prevalence of HIV-specific lesions was markedly reduced. Among 139
HIV+ patients, 86% presented any oral lesions with a prevalence of 76% of any periodontal diseases. Most periodontal lesions were classified as conventional gingivitis (28%) or periodontitis (30%). Prevalence for HIV-specific oral lesions was 29%, with a proportion of 9% of LGE, 3.6% of necrotizing and ulcerative gingivitis or periodontitis, 7% of oral candidiasis, 3.6% of oral hairy leukoplakia and single other lesions. Lack of oral hygiene determined by plaque formation and reduced CD4-counts with pronounced periodontal inflammation could be seen as risk factors for periodontal disease in HIV+ patients (Kroidl et al. 2005).

It has been suggested that HIV-infected patients are at risk of advanced periodontal disease with severe gingivitis, gingival recession, and alveolar bone loss (Alpagot et al. 2004). One of the most recent longitudinal studies evaluates periodontal probing depth (PD), clinical attachment level (CAL), and tooth loss from 584 HIV-seropositive and 151 HIV-seronegative women, recorded at 6-month intervals from 1995 to 2002. Adjusted longitudinal analysis showed that CD4 count and viral load had no consistent effects on PD or AL. Among HIV-infected women, a 10-fold increase in viral load was associated with a marginal increase in tooth loss. The progression of periodontal disease measured by PD and AL did not significantly differ between HIV-infected and HIV-uninfected women. The HIV-seropositive women lost more teeth (Alves et al. 2006).

Information on the microbiota associated with periodontitis in HIV-positive patients is controversial as several studies have shown that there is a similar microbial flora in both groups, and no difference in the distribution of black pigmented anaerobes could be observed (Patel et al. 2003; Botero et al. 2007a; Gonçalves Lde et al. 2007). It has been also shown that Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Aggregatibacter (Actinobacillus) actinomycetemcomitans, Eikenella corrodens and Campylobacter rectus may occur in diseased sites of HIV-positive patients and non-HIV-infected subjects with classical periodontitis (Patel et al. 2003). HIV-periodontitis seems also to be associated with elevated occurrence of Epstein–Barr virus type 2 (EBV-2), human herpes virus (HHV)-6 and EBV-1 were detected in severe adult periodontitis, Down’s syndrome periodontitis, periodontal abscesses, HIV-associated periodontitis and acute NUG (Slots and Contreras 2000; Saygun et al. 2004b).

Several risk factors for periodontitis in HIV+ individuals were identified, including age, smoking pack-years, viral load, F. nucleatum, P. intermedia, A. actinomycetemcomitans, neutrophil elastase, and β-glucuronidase in gingival crevicular fluid (Alpagot et al. 2004). It has been also reported that sites with high gingival crevice fluid levels of MMP-9 and TIMP-1 in HIV-positive patients are at significantly greater risk for progression of periodontitis (Alpagot et al. 2006).

In HIV-1-seropositive chronic periodontitis patients receiving periodontal therapy by conservative scaling and root planning and maintenance care, it has been showed that periodontal inflammatory parameters improved significantly under the immune reconstituting influence of highly active antiretroviral therapy (Jordan et al. 2006) (Table 2.4).

### 2.4.2 Herpesviruses

Studies during the past 10 years have associated herpesviruses with human periodontitis. The involvement of herpesviruses in the etiology of periodontal diseases is suggested by their presence in gingival tissue, gingival cervicular fluid and subgingival plaque, in the presence of periodontal disease (Cappuyns et al. 2005).

Of the approximately 120 identified different herpesviruses, eight major types are known to infect humans, namely, herpes simplex virus (HSV) type 1 and 2, varicella-zoster virus, EBV, Human cytomegalovirus (HCMV), (HHV)-6, HHV-7, and HHV-8 (Kaposi’s sarcoma virus) (Slots et al. 2005). Genomes of HCMV and EBV-1 were detected in severe adult periodontitis, localized juvenile periodontitis, generalized juvenile periodontitis, Papillon-Lefèvre syndrome periodontitis, Down’s syndrome periodontitis, periodontal abscesses, HIV-associated periodontitis and acute NUG (Slots and Contreras 2000; Saygun et al. 2004b).

Herpesviruses may cause periodontal pathogenesis as a direct result of virus infection and replication, or as a consequence of virally induced impairment of the periodontal immune defense, resulting in heightened virulence of resident bacterial pathogens (Slots 2005). An infectious disease model for the development of periodontitis based on herpesvirus-bacteria–host interactive responses was proposed by Slots (2005). Herpesvirus infection of periodontal sites may be important in a multistage pathogenesis by altering local host responses. Initially, bacterial infection of the gingival causes inflammatory cells to enter gingival tissue, with periodontal macrophages and T-lymphocytes harboring latent HCMV and periodontal B-lymphocytes harboring latent EBV (Contreras et al. 1999). Reactivation of herpesviruses from latency may occur spontaneously or during
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Country</th>
<th>Study population</th>
<th>Periodontal status</th>
<th>Main findings</th>
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<tbody>
<tr>
<td>Contreras et al. 2001</td>
<td>USA</td>
<td>21 HIV + patients and 14 HIV- negative patients</td>
<td>Chronic periodontitis</td>
<td>HIV-periodontitis seems to be associated with elevated occurrence of EBV-2, HHV-6 and herpesvirus coinfections compared to periodontitis in non-HIV-patients</td>
</tr>
<tr>
<td>Patel et al. 2003</td>
<td>South-Africa</td>
<td>20 HIV + patients and 20 HIV- negative patients</td>
<td>Chronic periodontitis</td>
<td>The results showed a significant prevalence of <em>P. gingivalis</em> and <em>Treponema denticola</em> among HIV-negative patients compared to HIV-positive patients. Odds ratio analysis revealed a statistically significant positive association between three of the 28 possible combinations in the HIV-positive group. They included <em>Prevotella nigrescens/Campylobacter rectus</em>, <em>P. nigrescens/P. gingivalis</em> and <em>P. nigrescens/T. denticola</em></td>
</tr>
<tr>
<td>Alves et al. 2006</td>
<td>USA</td>
<td>584 HIV-seropositive and 151 HIV-seronegative women</td>
<td>Chronic periodontitis</td>
<td>The progression of periodontal disease did not significantly differ between HIV-infected and HIV-uninfected women</td>
</tr>
<tr>
<td>Aas et al. 2007</td>
<td>USA</td>
<td>14 HIV + patients</td>
<td>Gingivitis, Chronic periodontitis, LGE</td>
<td>The classical putative periodontal pathogens, <em>Treponema denticola</em>, <em>Porphyromonas gingivalis</em> and <em>Tannerella forsythia</em> were below the limit of detection and were not detected. Species of <em>Gemella, Dialister, Streptococcus</em> and <em>Veillonella</em> were predominant</td>
</tr>
<tr>
<td>Botero et al. 2007a</td>
<td>Columbia</td>
<td>31 HIV + periodontitis patients, 32 HIV-negative periodontitis patients and 32 systemically and periodontally healthy patients</td>
<td>Chronic periodontitis</td>
<td>HIV + patients harbor higher levels of superinfecting microorganisms. <em>Pseudomonas aeruginosa, Enterobacter cloacae</em> and <em>Klebsiella pneumoniae</em> were identified</td>
</tr>
<tr>
<td>Gonçalves Lde et al. 2007</td>
<td>Brazil</td>
<td>72 subjects were distributed into two HIV-seropositive groups (37 chronic periodontitis and 35 periodontally healthy individuals) and two HIV-seronegative groups (49 chronic periodontitis and 51 periodontally healthy subjects)</td>
<td>Chronic periodontitis</td>
<td>Periodontal pathogens including <em>Tannerella forsythensis, Porphyromonas gingivalis, Prevotella nigrescens, Eubacterium nodatum, Fusobacterium nucleatum,</em> and <em>Selenomonas noxia</em> were more frequently detected in H-CP + subjects compared to H + CP + and controls. In contrast, <em>Enterococcus faecalis</em> and <em>Acinetobacter baumannii</em> were more commonly found in HIV-infected than in non-HIV-infected subjects</td>
</tr>
<tr>
<td>Grande et al. 2008</td>
<td>Brazil</td>
<td>50 HIV + patients and 50 HIV-negative patients</td>
<td>Chronic periodontitis</td>
<td>EBV-1 was more frequently recovered in oral sites of HIV-positive patients than in HIV-negative patients</td>
</tr>
</tbody>
</table>

*HSV-1* herpes simplex virus type 1; *EBV* Epstein–Barr virus; *HCMVEBV-1* human cytomegalovirus
periods of impaired host defense, resulting from immunosuppression, infection, physical trauma, hormonal changes, etc (Slots 2005). Herpesvirus-activating factors are also known risk factors/indicators for periodontal disease. Herpesviral activation leads to increased inflammatory mediator responses in macrophages, and probably also in connective tissue cells within the periodontal lesion (Slots 2005). After reaching a critical virus load, activated macrophages and lymphocytes may trigger a cytokine/chemokine “storm” of IL-1β, TNF-α, IL-6, prostaglandins, interferons, and other multifunctional mediators, some of which have the potential to propagate bone resorption. Herpesvirus induced immune impairment may also cause an upgrowth of resident gram-negative anaerobic bacteria, whose lipopolysaccharide together with HCMV, as discussed above, can induce cytokine and chemokine release from various mammalian cells, and may act synergistically in stimulating IL-1 gene transcription (Slots 2005; Botero et al. 2008a). In a vicious circle, the triggering of cytokine responses may activate latent herpesviruses, and in so doing, may further aggravate periodontal disease. Similarly, medical infections by HCMV can lead to increased susceptibility to bacterial and fungal infections and enhance the severity of existing microbial infections (Slots 2005). It is conceivable that herpesviruses rely on coinfection with periodontal bacteria to produce periodontitis and, conversely, periodontopathic bacteria may depend on viral presence for the initiation and progression of some types of periodontitis (Slots 2005). It was also showed that herpesvirus-infected periodontitis lesions tend to harbor elevated levels of classic periodontopathic bacteria, including Porphyromonas gingivalis, Dialister pneumosintes, Prevotella intermedia, Prevotella nigrescens, Campylobacter rectus, Treponema denticola and Actinobacillus (Aggregatibacter) actinomycetemcomitans (Slots 2005, 2007; Hanookai et al. 2000; Sunde et al. 2008; Saygun et al. 2004a; Kamma et al. 2001).

Table 2.5 resumes the occurrence of herpesviruses in periodontitis patients.

### 2.5 Transmission of Periodontal Pathogens

In periodontitis, as in other infectious diseases, knowledge of the source of pathogens and the route of infection is important for planning prevention strategies. It was suggested, based on the facts that periodontal pathogens cluster in families, that bacteria are transmitted between family members or that family members share susceptibility to colonisation of these bacteria (Asikainen and Chen 1999). Two types of transmission are recognized, vertical, that is, transmission from parent to offspring and horizontal, that is, passage of an organism between individuals outside the parent-offspring relationship (Haffajee and Socransky 1994) (Table 2.6).

In A. actinomycetemcomitans, but not in P. gingivalis, special clones associated with localized juvenile periodontitis have been identified. Vertical transmission of A. actinomycetemcomitans but not of P. gingivalis has been established. Most studies have shown that if children harbor A. actinomycetemcomitans, usually one or two parents harbor the same genotype. From these observations, it is assumed that the parent is the source of transmission. However, identical genotypes in family members are not 100% proof of transmission, as there is no infinite number of genotypes, and finding identical genotypes may have occurred by chance. The frequency of vertical transmission of A. actinomycetemcomitans is estimated to be between 30 and 60% based on detection of identical genotypes in children and parents (van Winkelhoff and Boutaga 2005).

Horizontal transmission of A. actinomycetemcomitans and P. gingivalis between spouses has been documented and may range between 14 and 60% for A. actinomycetemcomitans and between 30 and 75% for P. gingivalis. Transmission of A. actinomycetemcomitans between siblings has been suggested, but infection by the same source cannot be ruled out. Frequency of contact, number of organisms, oral health status, the resident microflora and immunological and genetic factors may determine whether a person will be permanently colonized by periodontal pathogens upon challenge. Although there is some limited evidence to show that cohabitation with a periodontitis patient influences the periodontal status of the spouse, substantially more information is needed to prove this hypothesis (van Winkelhoff and Boutaga 2005).

Since there is no evidence that periodontal pathogens would be disseminated in aerosols as, for example, respiratory pathogens, it is likely that the person-to-person transmission occurs via saliva and mucosal contact or an inanimate object. Therefore, suppression of the
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<tr>
<th>Author, year</th>
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<tbody>
<tr>
<td>Botero et al. 2007b</td>
<td>Colombia</td>
<td>30 periodontitis patients and 22 healthy controls</td>
<td>Aggressive periodontitis Chronic periodontitis</td>
<td>Plaque samples with paperpoints</td>
<td>HCMV detection was more prevalent in periodontally diseased subjects compared to healthy ones. Furthermore, in all groups, PD and CAL were increased in HCMV-positive sites. In the periodontitis groups, higher frequencies and levels of specific periodontopathic bacteria, such as <em>P. gingivalis, P. intermedia/ P. nigrescens</em>, and <em>E. corrodens</em>, were detected in HCMV-positive sites.</td>
</tr>
<tr>
<td>Botero et al. 2008b</td>
<td>Colombia</td>
<td>44 periodontitis patients and 24 healthy controls</td>
<td>Aggressive periodontitis Chronic periodontitis</td>
<td>GCF sample with paperpoints</td>
<td>Patients suffering from periodontitis had a higher frequency of HCMV as detected by nested PCR (79.5%) and real-time PCR (47.7%) in comparison to healthy subjects (25% nested PCR, 4.1% real-time PCR).</td>
</tr>
<tr>
<td>Chalabi et al. 2008</td>
<td>Iran</td>
<td>61 periodontitis patients and 40 healthy controls</td>
<td>Chronic periodontitis</td>
<td>Plaque samples with curette</td>
<td>Prevalence of EBV-1, EBV-2 and CMV among patients with periodontitis were 73.8, 4.9 and 59%; respectively.</td>
</tr>
<tr>
<td>Contreras and Slots 1996</td>
<td>USA</td>
<td>27 periodontitis patients</td>
<td>Adult periodontitis</td>
<td>GCF sample with paperpoints</td>
<td>89% subjects yielded at least one of the five test viruses from deep periodontal pockets, whereas only 56% showed viruses from shallow periodontal sites. Viral coinfection occurred more frequently in deep than in shallow periodontal sites. HCMV was detected with higher frequency in deep than in shallow periodontal sites.</td>
</tr>
<tr>
<td>Contreras and Slots 1998</td>
<td>USA</td>
<td>6 periodontitis patients</td>
<td>Adult and juvenile periodontitis</td>
<td>GCF sample with paperpoints</td>
<td>Subgingival HCMV DNA was more present in periodontitis (89%) than in 22% gingivitis sites (22%), suggesting that active HCMV replication can occur in periodontitits sites.</td>
</tr>
<tr>
<td>Hanookai et al. 2000</td>
<td>USA</td>
<td>19 Trisomy 21 patients and 20 healthy controls</td>
<td>Mild, moderate and advanced periodontitis</td>
<td>Plaque samples with curette</td>
<td>Of 19 Trisomy 21 periodontitis lesions, 32% were positive for EBV-1, 26% were positive for HCMV, 16% were positive for HSV, and 11% showed viral coinfection. Of 19 shallow periodontal sites, only one revealed HCMV. Subgingival debridement did not reduce genomic herpesvirus presence.</td>
</tr>
<tr>
<td>Idesawa et al. 2004</td>
<td>Japan</td>
<td>33 periodontitis patients and 20 healthy controls</td>
<td>Chronic periodontitis</td>
<td>Saliva</td>
<td>Salivary levels of EBV was detected in 48.5% periodontitis patients and in 15% healthy subjects. The baseline mean values for BOP in EBV-positive patients were significantly higher than those in EBV-negative patients.</td>
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### Table 2.5 (continued)

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<tr>
<td>Kamma et al.</td>
<td>Greece</td>
<td>16 periodontitis patients</td>
<td>Aggressive periodontitis</td>
<td>Plaque samples with paperpoints</td>
<td>HCMV was detected in 59.4% of active and in 12.5% of stable sites, EBV-1 in 43.8% of active and in 12.5% of stable sites, HSV in 34.5% of active and in 9.4% of stable sites, and coinfection with any of the three test herpesviruses in 43.8% of active and in 3.1% of stable sites. All periodontitis sites showing herpesvirus coinfection and all but one site showing <em>P. gingivalis</em> and <em>D. pneumoniae</em> coinfection revealed bleeding upon probing.</td>
</tr>
<tr>
<td>Kubar et al.</td>
<td>Turkey</td>
<td>16 periodontitis patients and 15 healthy control</td>
<td>Aggressive periodontitis</td>
<td>Plaque samples with curette</td>
<td>HCMV was detected in 68.8% of aggressive periodontitis lesions but not in any of the periodontally healthy study sites.</td>
</tr>
<tr>
<td>Kubar et al.</td>
<td>Turkey</td>
<td>20 periodontitis patients</td>
<td>Aggressive periodontitis Chronic periodontitis</td>
<td>Plaque samples with curette</td>
<td>HCMV DNA was detected in 78% of subgingival and 33% of gingival tissue samples from aggressive periodontitis lesions, but only in 46% of subgingival and 9% of gingival tissue samples from chronic periodontitis lesions. In aggressive periodontitis, HCMV subgingival and gingival tissue counts were positively correlated with periodontal probing depth and CAL at sample sites. EBV DNA was identified in 89% of subgingival and 78% of gingival tissue samples from aggressive periodontitis lesions, but only in 46% of both subgingival and gingival tissue samples from chronic periodontitis lesions. In aggressive periodontitis, positive correlations were found for EBV subgingival counts and periodontal probing depth at sample sites and for EBV gingival tissue counts and whole mouth mean gingival index. HCMV–EBV coinfection was revealed in 78% of aggressive periodontitis lesions but only 27% of chronic periodontitis lesions.</td>
</tr>
<tr>
<td>Ling et al.</td>
<td>USA</td>
<td>20 periodontitis patients</td>
<td>Chronic periodontitis</td>
<td>Plaque samples with paperpoints</td>
<td>The prevalence of HSV or HCMV was significantly higher in the subgroups that had lower plaque index. However, the prevalence of HSV was significantly higher in the subgroup that had higher gingival index, positive BOP, deeper PD or higher PAL. Moreover, the prevalence of EBV-1 was significantly higher in the subgroup that had higher PD. Coinfection of HSV and HCMV was significantly associated with the sites that had higher gingival index or positive BOP. Coinfection of any two herpesviruses was also associated with higher PD or higher PAL.</td>
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</tr>
</thead>
<tbody>
<tr>
<td>Parra and Slots</td>
<td>USA</td>
<td>56 gingivitis and periodontitis patients</td>
<td>Advanced periodontitis and gingivitis</td>
<td>GCF sample with paperpoints</td>
<td>Cytomegalovirus was detected in 60% of the periodontitis patients, EBV in 30%, HSV in 20%, human papillomavirus in 17% and HIV in 7%. Forty percent of the periodontitis patients revealed coinfection by two to five viruses. Only 31% of the gingivitis subjects showed a positive viral identification in crevicular fluid, and infected individuals only revealed human HCMV.</td>
</tr>
<tr>
<td>Rotola et al.</td>
<td>Italy</td>
<td>24 periodontitis patients, 13 healthy controls</td>
<td>Aggressive periodontitis (11) Chronic periodontitis (13)</td>
<td>gingival biopsies</td>
<td>HHV-7 was detected in 91.7% of periodontitis patients and in 61.5% of healthy subjects, EBV in 50.0% samples of periodontitis patients and 7.7% of H subjects and HCMV only in one sample from H group. EBV was more frequently detected in biopsies from affected sites (50.0%) than from nonaffected sites (16.7%). HHV-7 transcription was detected in 15.4% of affected and 15.4% of nonaffected sites.</td>
</tr>
<tr>
<td>Saygun et al.</td>
<td>Turkey</td>
<td>30 periodontitis patients, 21 healthy controls</td>
<td>Chronic periodontitis</td>
<td>Plaque samples with paperpoints</td>
<td>HCMV was detected in 44.3% of chronic periodontitis patients and 14.3% of healthy persons; EBV-1 in 16.7% of chronic periodontitis patients and 14.3% of healthy persons; and HSV in 6.7% of chronic periodontitis patients and in no healthy persons. HCMV and EBV-1 detected and undetected sites in patients with periodontitis showed statistically significant differences in sampling clinical depth and sampling CAL.</td>
</tr>
<tr>
<td>Saygun et al.</td>
<td>Turkey</td>
<td>18 periodontitis patients, 16 healthy controls</td>
<td>Aggressive advanced periodontitis</td>
<td>Plaque samples with curette</td>
<td>HCMV, EBV-1 and HSV-1 were each detected in 72–78% of the aggressive periodontitis patients. HSV-2 occurred in 17% of the periodontitis patients. EBV-1 was detected in one periodontally healthy subject. HCMV, EBV-1 and HSV-1 were positively associated with \textit{P. gingivalis, P. intermedia, T. forsythia} and \textit{C. rectus}, but not with \textit{A. actinomycetemcomitans}. HSV-2 was not associated with any test bacteria.</td>
</tr>
<tr>
<td>Saygun et al.</td>
<td>Turkey</td>
<td>15 periodontitis patients, 15 healthy controls</td>
<td>Aggressive periodontitis (9) Chronic periodontitis (6)</td>
<td>Plaque samples with curette</td>
<td>HCMV was detected in eight periodontitis lesions and in one normal periodontal site, EBV was detected in nine periodontitis lesions and in two normal periodontal sites. Correlations were found between counts of HCMV and EBV, between counts of HCMV and \textit{P. gingivalis, T. forsythia} and \textit{C. rectus}, and between counts of EBV and \textit{P. gingivalis} and \textit{T. forsythia}. HCMV and EBV virus counts were also positively associated with the level of PAL, PD and BOP.</td>
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2.5 Transmission of Periodontal Pathogens

### Table 2.5 (continued)

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<tr>
<th>Author, year</th>
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<th>Periodontal status</th>
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<th>Main findings</th>
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<tr>
<td>Slots et al. 2002</td>
<td>Greece</td>
<td>16 periodontitis patients</td>
<td>Aggressive periodontitis</td>
<td>Plaque samples with paperpoints</td>
<td>HCMV, EBV-1, HSV, <em>D. pneumosintes</em> and <em>P. gingivalis</em> were detected more frequently in periodontitis active than in periodontitis stable sites. HCMV was significantly positively associated with <em>D. pneumosintes</em>. HCMV was positively associated with PD, average percentage bone loss, disease-active periodontitis and gender. EBV-1 was positively associated with PD, PAL, disease-active periodontitis and total number of teeth. HSV was positively associated PD, PAL and disease-active periodontitis</td>
</tr>
<tr>
<td>Slots et al. 2003</td>
<td>Greece</td>
<td>16 periodontitis patients</td>
<td>Aggressive periodontitis</td>
<td>Plaque samples with paperpoints</td>
<td>HCMV and HSV were both significant predictors of the presence of subgingival <em>P. gingivalis</em></td>
</tr>
<tr>
<td>Sunde et al. 2008</td>
<td>USA</td>
<td>25 periodontitis patients</td>
<td>“Refractory” marginal periodontitis</td>
<td>GCF sample with paperpoints</td>
<td>Of 11 deep periodontal samples, 72% showed HCMV, 63% showed Epstein–Barr virus type 1 (EBV-1), 9% showed EBV type 2, 54% showed HSV and 72% showed viral coinfection. Of 11 shallow periodontal samples, 18% showed HCMV, 18% showed EBV-1, 9% showed HSV and 18% showed viral coinfection</td>
</tr>
<tr>
<td>Ting et al. 2000</td>
<td>USA</td>
<td>11 periodontitis patients</td>
<td>Localized juvenile periodontitis</td>
<td>GCF sample with paperpoints</td>
<td>Of 11 deep periodontal samples, 72% showed HCMV, 63% showed Epstein–Barr virus type 1 (EBV-1), 9% showed EBV type 2, 54% showed HSV and 72% showed viral coinfection. Of 11 shallow periodontal samples, 18% showed HCMV, 18% showed EBV-1, 9% showed HSV and 18% showed viral coinfection</td>
</tr>
<tr>
<td>Watanabe et al. 2007</td>
<td>Brazil</td>
<td>30 periodontitis patients</td>
<td>Aggressive periodontitis</td>
<td>GCF samples by paperpoints</td>
<td>77% of patients were positive for EBV-1, while only 6% were positive for HCMV. A positive association between EBV-1 and periodontal lesions was revealed. 57% of periodontitis sites were positive for EBV-1, whereas 30% of gingivitis sites were positive</td>
</tr>
<tr>
<td>Wu et al. 2007</td>
<td>Chinese</td>
<td>143 periodontitis patients, 65 gingivitis patients and 76 healthy controls</td>
<td>Chronic periodontitis Gingivitis</td>
<td>Plaque samples with paperpoints</td>
<td>HCMV was detected in 79.0% of chronic periodontitis patients, 78.5% gingivitis patients, and 76.3% periodontally healthy individuals, while EBV was found in 63.6, 32.3, and 30.3% of the three groups of subjects, respectively. HCMV gB-II infection and HCMV gB-II coinfection with EBV-1 are closely associated with periodontal tissue inflammation and destruction</td>
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(continued)
organisms in saliva may prevent their spread among individuals (Asikainen and Chen 1999). The effects of prevention of transmission of *A. actinomycetemcomitans* and *P. gingivalis* have not been studied so far. For *A. actinomycetemcomitans*, screening for and prevention of transmission of specific virulent clones may be feasible and effective in preventing some forms of periodontal disease. *P. gingivalis* is usually recovered from diseased adult subjects, and transmission of this pathogen seems largely restricted to adult individuals.
Horizontal transmission of *P. gingivalis* may therefore be controlled by periodontal treatment involving elimination or significant suppression of the pathogen in diseased individuals, and by a high standard of oral hygiene (van Winkelhoff and Boutaga 2005).

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