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Etiology and Pathogenesis of Periodontal Diseases

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According to the most recent classification that resulted from a 1999 international workshop [1], diseases of the periodontium comprise a long list of conditions involving the supporting structures of the tooth. The two most prevalent and most investigated periodontal diseases are dental plaque–induced gingivitis and chronic periodontitis. Aggressive periodontitis, which encompasses several previous clinical entities such as localized juvenile periodontitis (currently termed localized aggressive periodontitis, LAP), generalized juvenile periodontitis and rapidly progressing periodontitis (now collectively classified as generalized aggressive periodontitis), periodontitis associated with systemic diseases, and necrotizing ulcerative periodontal diseases (ie, necrotizing ulcerative gingivitis and periodontitis), round out the list of the most significant and common periodontal diseases. The last 10 to 15 years have seen the emergence of several important new findings and concepts regarding the etiopathogenesis of periodontal diseases. These findings include the recognition of dental bacterial plaque as a biofilm, identification and characterization of genetic defects that predispose individuals to periodontitis, host-defense mechanisms implicated in periodontal tissue destruction, and the interaction of risk factors with the host defenses and bacterial plaque. This article reviews current aspects of the etiology and pathogenesis of periodontal diseases.

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Etiology and pathogenesis of periodontal diseases

Gingivitis and periodontitis are inflammatory conditions of infectious nature [2]. The unequivocal role of dental bacterial plaque in the development of these diseases was established almost 40 years ago [3–7]. Gingivitis is a reversible inflammatory reaction of the marginal gingiva to plaque accumulation, whereas periodontitis is a destructive, nonreversible condition resulting in loss of tooth connective-tissue attachment to bone, which ultimately leads to loss of the involved teeth. Existing evidence indicates that gingivitis precedes the onset of periodontitis; however, not all gingivitis cases develop into periodontitis [5,8,9]. The reason for this is that accumulation of plaque bacteria is necessary but not sufficient by itself for the development of periodontitis: a susceptible host is necessary [5,9,10].

The role of dental plaque

The presence of bacteria in the oral cavity has been known since the time of Anton von Leeuwenhoek, who described the presence of ''animalcules'' in dental plaque. The bacterial etiology of periodontal diseases has been explored for over 100 years, evolving along with technologic advances in identification and characterization. Although early studies indicated that periodontal diseases occurred in response to plaque mass (nonspecific plaque hypothesis), current thinking implicates specific microbial species in disease causation (specific plaque hypothesis) [11,12]. This bacterial etiology of periodontal disease is strongly supported by clinical studies that have reported that mechanical and chemical antibacterial treatment can prevent or treat gingivitis and periodontitis [12,13].

The identification of specific causative species, or periodontopathogens, has been hampered by some of the unique features of periodontal diseases. The foremost of these features is that disease occurs in a site already colonized by a bacterial population. Thus, disease might be caused by overgrowth of one or more species in the resident population or by colonization by exogenous pathogens. For example, it has been shown that *Capnocytophaga* spp are seen in high levels before the onset of gingivitis, whereas *Prevotella* spp are detected in areas with established gingivitis. Thus, it may be interpreted that *Capnocytophaga* spp are more likely to be etiologic agents and *Prevotella* spp may be present as a consequence of the disease process [14]. Colonization by exogenous pathogens is thought to contribute to the episodic nature of disease progression [15]; that is, not all sites with baseline-attachment loss demonstrate the same rate of disease progression or disease activity at the same time points.

Many methods have been used to study the composition of plaque bacteria. Initial studies were based on cultivation and microscopic visualization. Research studies using detection systems based on specific antibodies were useful in determining the presence and levels of species of

interest. With the advent of molecular methods, however, the diversity of the oral flora has been extensively explored. Studies by Kroes et al [16], Paster et al [17], and Choi et al [18] have revealed that over half of the plaque accumulated is composed of heretofore uncultivated species. Culture-based studies have not been able to explore the diversity of this polymicrobial infection. Thus, it is possible that as-yet-undetected species are responsible for disease.

One of the most significant recent developments in the understanding of periodontal disease etiology is the recognition of dental plaque as a biofilm. A biofilm is defined as single cells and microcolonies enclosed in a highly hydrated, predominantly anionic exopolymer matrix [19]. These sessile cells behave in profoundly different ways from their free-floating (planktonic) counterparts.

Dental plaque as a biofilm—development

Research on plaque development has shown that oral bacteria colonize nonshedding hard surfaces and shedding soft tissue surfaces. The physical and morphologic characteristics of these surfaces create different ecosystems or niches with distinct bacterial profiles. These niches are the result of a dynamic equilibrium that exists between the adhesion forces of microorganisms and swallowing and mastication forces, salivary and crevicular flow, and oral hygiene measures.

Bacterial adhesion to oral surfaces is a function of bacterial surface characteristics and the receptivity of the host epithelium. Studies on bacterial colonization of other niches in the body have shown that epithelial cells of individuals susceptible to infection harbor as many as five times more pathogenic bacteria than resistant individuals [20]. Studies using animal models suggest that the same might be true of periodontal disease [21].

Plaque formation follows several distinct phases, beginning with adsorption onto the tooth surface of a conditioning film derived from bacterial and host molecules that forms immediately following tooth eruption or tooth cleaning [22]. This adsorption is followed by passive transport of bacteria mediated by weak, long-range forces of attraction. Covalent and hydrogen bonds create strong, short-range forces that result in irreversible attachment [23]. The primary colonizers form a biofilm by autoaggregation (attraction between same species) and coaggregation (attraction between different species). Coaggregation results in a functional organization of plaque bacteria and formation of different morphologic structures such as corncobs and rosettes. The microenvironment now changes from aerobic/capnophilic to facultative anaerobic. The attached bacteria multiply and secrete an extracellular matrix, which results in a mature mixed-population biofilm [24]. Transmission occurs from other sites, leading to incorporation of new members into the biofilm and the formation of a climax community.

A biofilm environment confers certain properties to bacteria that are not seen in the planktonic state, a fact that explains the importance of recognizing dental plaque as a biofilm and not as bacteria in the planktonic state. In the following sections, some of the properties of a biofilm are reviewed.

Cell–cell communication. An important characteristic seen only in biofilm-associated bacteria is quorum sensing, or cell density–mediated gene expression. This process is mediated by two groups of compounds known as autoinducer 1 and autoinducer 2. Gram-positive and gram-negative cells secrete molecules known as autoinducer-2, which is encoded by the *luxS* gene [25]. In *Streptococcus gordonii*, *luxS* turns on expression of *sspA* and *sspB* genes, which encode for a protein that provides a binding site for the major fimbriae of *Porphyromonas gingivalis* [26–29]. Thus, *Porphyromonas gingivalis*, a secondary colonizer, is capable of preferential binding in the presence of *S gordonii*, an early colonizer.

Gene transfer. Biofilm-associated bacteria communicate with each other by way of horizontal gene transfer. In *S mutans*, quorum sensing is mediated by competence-stimulating peptide. Genes of the competence-stimulating peptide signaling system (*comC*, *comD*, *comE*, and *comX*) are responsible for multiple functions: biofilm formation, competence (ability to accept foreign DNA), and acid tolerance [30,31].

Antimicrobial resistance. The biofilm provides a protected environment against antimicrobial agents. The biofilm acts as a barrier to diffusion due to the presence of neutralizing enzymes (β-lactamase, IgA protease) and a diffusion-resistant matrix [32]. Cells in the biofilm can develop antibiotic resistance from horizontal gene transfer and mutations and by expressing efflux pumps. Penicillin [33] and tetracycline [34] resistance occurs among bacteria by conjugative transfer of mobile genetic elements. Further, cells in a biofilm have a slower rate of cell division and might therefore not be as susceptible to antibiotics as actively dividing planktonic cells. This resistance provided by biofilm living must be taken into account when selecting antibiotics. In vitro antibiotic susceptibility tests are typically conducted on planktonic cells whose behavior is significantly different from that of biofilm-associated cells. Thus, it has been argued that ''biofilm inhibitory concentration'' is a more realistic estimate of antimicrobial activity than the current measures [35].

Regulation of gene expression. Biofilm living has been shown to regulate gene expression in certain bacteria. For example, exposure of *S gordonii* to saliva results in the induction of genes (*sspA*/*B*) that mediate host-surface binding and coaggregation with *Porphyromonas gingivalis* and *Actinomyces*

spp [36,37]. Similarly, genes encoding glucan (*gtf*) and fructan (*ftf*) synthesis are differentially regulated in biofilm-associated *S mutans* [38].

Bacterial antigens and virulence factors in the biofilm

Recent studies have focused on "virulence factors" associated with putative periodontopathogens. A virulence factor is any microbial component that is necessary for causing disease in the host. These factors may be essential for host colonization or may be "true" antigens capable of causing an immune response by the host. In vitro studies of biofilms have enabled the study of the antigenic potential of biofilms [39].

Virulence factors can be differentially expressed in response to the environment. *Actinobacillus actinomycetemcomitans*, for example, produces leukotoxin, a 116-kd protein antigen capable of immunomodulation [40]. Leukotoxin causes apoptosis (programmed cell death of leukocytes), the first line of defense against bacterial invasion. Because the organism grows optimally at pH 7.0 to 8.0, it is suggested that the more virulent strains of *Actinobacillus actinomycetemcomitans* are present on the surface of subgingival plaque [40]. Similarly, *Porphyromonas gingivalis* strain W50 shows maximal growth and proteolytic activity in conditions of pH 7.0 to 8.0 and hemin excess, suggesting it becomes more virulent in an inflamed gingival crevice [41]. Among the specific virulence factors associated with putative periodontopathogens are the following molecules (Table 1).

Lipopolysaccharide. The outer membranes of most gram-negative bacteria display repeating polysaccharide units attached to the O-side chains of a lipid component (lipopolysaccharide). Lipopolysaccharide is highly antigenic and capable of inducing the release of inflammatory mediators and cytokines such as prostaglandin E_2, interleukin (IL)-1α, IL-1β, tumor necrosis factor-α (TNF-α), and IL-8 through CD14 and Toll-like receptor–mediated activation of several host cells. The structure of lipopolysaccharide also allows classification of bacteria based on its structure. For example, *Actinobacillus actinomycetemcomitans* can be classified into six distinct serotypes of varying virulence based on lipopolysaccharide structure [42]. Unlike other bacteria, lipopolysaccharide of *Porphyromonas gingivalis* activates the cytokine cascade through Toll-like receptors, perhaps because of its unique three-dimensional structure [43].

Heat shock proteins. When eukaryotic and bacterial cells are exposed to environmental stress (eg, temperature, pH, redox potential), they synthesize stress proteins such as heat shock proteins [44]. These proteins act as molecular chaperones in the assembly and folding of proteins and thus protect the cell from the damaging effects of environmental stress. Heat shock protein homologs such as GroEL, GroES, DnaK, and HtpG have been studied in oral bacteria. Heat shock proteins from *Actinobacillus actinomycetemcomitans* stimulate osteoclast activation and epithelial

Table 1
Bacterial virulence factors and their effects on the host

Factor	Produced by	Effects on host cells
Lipopoly-saccharide	Gram negative bacteria including *Actinobacillus actinomycetemcomitans, Treponema denticola, Porphyromonas gingivalis, Tannerella forsythia*	Increases cytokine release from PMNs, macrophages, fibroblasts Induces nitric oxide secretion in macrophages Increases differentiation of osteoclast precursors Activates osteoclasts Supports osteoclast survival Stimulates T-helper cell proliferation
Heat shock proteins	*Actinobacillus actinomycetemcomitans, Tannerella forsythia, Porphyromonas gingivalis, Campylobacter rectus, Fusobacterium nucleatum, Streptococcus mutans, Prevotella* spp, *Capnocytophaga* spp	Induce gingival epithelial cell proliferation in low doses Osteolytic activity PDL epithelial cell proliferation Molecular mimicry between bacterial and host heat shock proteins, leading to autoimmune response
Extracellular proteolytic enzymes	*Tannerella forsythia, Porphyromonas gingivalis, Treponema denticola*	Degrade fibrinogen, fibronectin, albumin, laminin Hydrolyze collagen IV, IgG, IgA
Fimbriae	*Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis*	Important in colonization Role in invasion of host cells
Outer membrane proteins	*Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis*	Enhance innate immune response through cytokines Suppress proliferation of T and B cells Suppress proliferation of fibroblasts, monocytes, osteoblasts
Leukotoxin	*Actinobacillus actinomycetemcomitans*	Promotes apoptosis of T cells, natural killer cells, PMNs (in low concentration) Causes cell death by necrosis (in high concentration)
Flagellum	*Treponema denticola*	Role in adherence, due to fibronectin binding
Capsule	*Porphyromonas gingivalis*	Increases resistance to phagocytosis by PMNs Inhibits fibroblast attachment to root surface

Abbreviations: PDL, periodontal ligament; PMNs, polymorphonuclear leukocytes.

proliferation at low concentrations and are cytotoxic at high doses [45]. Microbial heat shock proteins are highly immunogenic and have been shown to be associated with autoimmune diseases such as rheumatoid arthritis and atherosclerosis. It has been suggested that chronic infections such as periodontitis may result from prolonged exposure to heat shock proteins, thereby promoting autoimmune disease. The evidence, however, is tenuous, as seen with *Porphyromonas gingivalis* GroEL [46,47].

Fimbriae. Fimbriae are found in oral bacteria such as *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. They are long

protein filaments, present singly or in bundles on the surface of cells. The major component is fimbrillin, a highly antigenic protein encoded by *fimA* in *Porphyromonas gingivalis* and *flp* in *Actinobacillus actinomycetemcomitans*. In both bacteria, fimbriae are thought to be important in colonization because fimbrial-deficient mutants show reduced ability to bind and invade epithelial cells and fibroblasts [48]. Fimbriae-mediated epithelial invasion stimulates expression of host cell adhesion molecules such as intercellular adhesion molecule, vascular cell adhesion molecule, P-selectin, and E-selectin, thus inducing a massive leukocytic response at the site. *Porphyromonas gingivalis* fimbriae also stimulate IL-1α, IL-1β, TNF-α, and granulocyte-macrophage colony–stimulating factor, leading to bone resorption.

Extracellular proteolytic enzymes. *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*, and other oral bacteria produce proteolytic enzymes often displayed on their cell surfaces. Dentilisin (*Treponema denticola*), PrtH (*Tannerella forsythia*), and RgpA, RgpB, and Kgp (*Porphyromonas gingivalis*) are the best-characterized enzymes in this group [49]. The RgpA/B and Kgp proteinases, major virulence factors of *Porphyromonas gingivalis*, degrade fibrinogen, fibronectin, laminin, adhesion molecules, and several types of collagen [50–52]. *Tannerella forsythia* with the *prtH* genotype is found to be more common in chronic periodontitis than in health. Dentilisin has been shown to hydrolyze fibrinogen, albumin, laminin, collagen type IV, IgG, and IgA.

Although there is compelling evidence that bacteria and bacterial products present a challenge to the host defense system, there is no single bacterial product that induces all the changes observed in periodontal disease. Henderson and coworkers [53] proposed that bacterial modulins are responsible for immunomodulation of the host cells. Modulins are bioactive bacterial compounds such as exotoxins, endotoxins, and metabolic end products produced by commensal and pathogenic organisms that can induce host cytokine networks. What is evident from the preceding description of the various virulence factors is that bacteria can cause direct tissue damage; however, by triggering several host cells to release inflammatory cytokines and other mediators (see later discussion), bacteria can also cause indirect tissue damage. It seems that the host response to the bacteria renders most of the damage seen in periodontal disease.

Specific microorganisms associated with periodontal health and disease

Different periodontal diseases have somewhat unique profiles of associated bacteria (Table 2). This characteristic and the fact that disease occurs in sporadic bursts in the mouth strengthens the evidence for the role of specific microorganisms in disease causation and progression.

Table 2
Bacterial species associated with periodontal health and disease

Condition	Associated microorganisms	Strength of evidence	Techniques used
Periodontal health	Gram (+) anaerobe		
	Atopobium rimae	CS	m
	Gram (+) facultative		
	Streptococcus sanguis	CS, LS	C, M, I, c, m
	Streptococcus mitis	CS, LS	C, M, I, c, m
	Gram (−) anaerobe		
	Bacteroides sp oral clone BU063	CS	m
	Veillonella spp	CS, LS	C, M, I, c, m
	Gemella spp	CS, LS	C, M, I, c, m
	Capnocytophaga spp	CS, LS	C, M, I, c, m
Gingivitis	Gram (+) anaerobe		
	Actinomyces viscosus	CS	C, M, I, c
	Peptostreptococcus micros	CS, LS	C, M, I, c
	Gram (+) facultative		
	Streptococcus spp	CS, LS	C, M, I, c
	Gram (−) anaerobe		
	Campylobacter gracilis	CS, LS	C, M, I, c
	Fusobacterium nucleatum	CS, LS	C, M, I, c
	Prevotella intermedia	CS, LS	C, M, I, c
	Veillonella parvula	CS, LS	C, M, I, c
Chronic periodontitis	Gram (+) anaerobe		
	Peptostreptococcus micros	CS	C, M, I, c, m
	Gram (−) anaerobe		
	Porphyromonas gingivalis	CS, LS	C, M, I, c, m
	Tannerella forsythia	CS, LS	C, M, I, c, m
	Prevotella intermedia	CS, LS	C, M, I, c, m
	Campylobacter rectus	CS, LS	C, M, I, c, m
	Eikenella corrodens	CS	C, M, I, c, m
	Fusobacterium nucleatum	CS, LS	C, M, I, c, m
	Actinobacillus actinomycetemcomitans	CS	C, I, c, m
	Treponema spp	CS, LS	C, M, I, c, m
	Filifactor alocis	CS	C, I, c, m
	Megasphaera sp oral clone BB166	CS	m
	Deferribacteres sp oral clone BH017	CS	m
	Desulfobulbus sp oral clone R004	CS	m
	Bacteroides sp oral clone AU126	CS	m
Aggressive periodontitis	Gram (−) anaerobe		
	Actinobacillus actinomycetemcomitans	CS, LS	C, c, m
	Porphyromonas gingivalis	CS, LS	C, I, c, m
	Campylobacter rectus	CS	C
	Eikenella corrodens	CS	C
Acute necrotizing gingivitis	Gram (−) anaerobe		
	Treponema spp	CS	C, M, m
	Prevotella intermedia	CS	m
	Rothia dentocariosa	CS	m
	Fusobacterium spp	CS	m
	Achromobacter spp	CS	m

Table 2 (*continued*)

Condition	Associated microorganisms	Strength of evidence	Techniques used
	Propionibacterium acnes	CS	m
	Capnocytophaga spp	CS	m
Periodontal abscess	Gram (+) anaerobe		
	Peptostreptococcus micros	CS	C
	Gram (−) anaerobe		
	Fusobacterium nucleatum	CS	C
	Prevotella intermedia	CS	C
	Tannerella forsythia	CS	C
	Campylobacter rectus	CS	C

Abbreviations: c, checkerboard; C, culture; CS, cross-sectional studies; I, immunodetection; LS, longitudinal studies; m, molecular methods; M, microscopy.

Periodontal health

Bacteria that are associated with periodontal health include primary or early colonizers such as *S sanguis*, *S mitis*, *Gemella* spp, *Atopobium* spp, *Fusobacterium nucleatum*, and *Capnocytophaga* spp [54–56]. Species belonging to the genera *Veillonella*, *Streptococcus*, and *Capnocytophaga* are thought to be beneficial to the host [55]. Recent molecular analysis has shown the presence of certain uncultivated species such as *Bacteroides* oral clone BU063 to be strongly associated with periodontal health [57].

Gingivitis

Gram-positive species (eg, *Streptococcus* spp, *Actinomyces viscosus*, *Peptostreptococcus micros*) and gram-negative species (eg, *Campylobacter gracilis*, *F nucleatum*, *Prevotella intermedia*, *Veillonella*) have been associated with gingivitis [13,58,59]. Pregnancy-associated gingivitis, however, appears to have a microflora with a high proportion of *Prevotella intermedia* [60].

Chronic periodontitis

The bacterial profile of chronic periodontitis has been explored in cross-sectional and longitudinal studies. The effect of various treatment methods in changing the microbial ecology has also been investigated. *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Campylobacter rectus*, *Eikenella corrodens*, *F nucleatum*, *Actinobacillus actinomycetemcomitans*, *Peptostreptococcus micros*, and *Treponema* spp have been most commonly found to be associated with chronic periodontitis. *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Campylobacter rectus*, and *F nucleatum* have been reported at higher levels in sites with active disease or with progressing disease [61–64]. Clinical resolution of disease following treatment is also associated with a decrease in the levels of these species. More recent molecular approaches have also found previously uncultivated

bacterial species such as *Desulfobulbus* sp oral clone R004, *Deferribacteres* sp oral clones BH017 and D084, and *Bacteroides* sp oral clone AU126 to be associated with periodontitis [17,54].

Localized aggressive periodontitis

Studies have implicated *Actinobacillus actinomycetemcomitans* as an important organism in the etiology of LAP [65–67]. This species has been found as the predominant cultivable species in as many as 90% of sites with LAP. It should be noted, however, that not all studies support the association of *Actinobacillus actinomycetemcomitans* in aggressive periodontitis. This organism is not always found in disease sites; further, it has also been found in healthy children, albeit in low numbers, suggesting that it is a member of the healthy microbial flora [68]. Other species such as *Porphyromonas gingivalis*, *E corrodens*, and *Campylobacter rectus* have been found in high levels in certain cases of LAP [69]. Viruses such as Epstein Barr virus and human cytomegalovirus have also been associated with this disease [70].

Generalized aggressive periodontitis

The microbial etiology of generalized aggressive periodontitis is not as well defined as the other forms of periodontal diseases due to changes in disease nomenclature over time. The disease now encompasses entities such as periodontosis, prepubertal periodontitis, and rapidly progressing periodontitis. Nevertheless, available evidence suggests that the bacterial profile of generalized aggressive periodontitis is not significantly different from that of chronic periodontitis [71–73].

Necrotizing ulcerative gingivitis

The bacterial flora of necrotizing ulcerative gingivitis has been demonstrated to be composed, for the most part, of fusobacteria and spirochetes. Recent studies have isolated previously unsuspected spirochetes (eg, *Treponema putidum*, a proteolytic treponeme) from lesions of necrotizing ulcerative gingivitis [74]. Other bacteria reported in these lesions include *Rothia dentocariosa*, *Treponema* spp, *Achromobacter* spp, *Propionibacterium acnes, Capnocytophaga* spp, and *Prevotella intermedia* [56].

Periodontal abscess

A periodontal abscess is a localized purulent infection within the tissues adjacent to the periodontal pocket. *F nucleatum, Prevotella intermedia, Peptostreptococcus micros, Tannerella forsythia, Campylobacter rectus, and Porphyromonas gingivalis* have been recovered from these lesions [75,76].

The role of host susceptibility

As mentioned earlier, plaque bacteria are necessary but not sufficient for the development of periodontitis: a susceptible host is necessary. This aspect of periodontal pathogenesis is addressed in the following paragraphs.

Gingivitis

Although development of gingivitis after plaque accumulation appears to be a universal finding, the rate or speed of development and the degree of the clinical inflammatory response is variable between individuals, even under similar plaque accumulation conditions [77]. In contrast to the voluminous literature on periodontitis, there are few studies addressing potential host-dependent variations in susceptibility to gingivitis [77,78]. Based on studies using the experimental gingivitis model, one can estimate that approximately 13% of all individuals represent a ''resistant'' group [77,79,80]. Several factors have been shown to modulate the clinical expression of gingival inflammation in response to plaque accumulation. Some factors result in exacerbated gingival response to plaque, including metabolic factors such as puberty and pregnancy, genetic factors such as Down syndrome, nutritional factors such as vitamin C deficiency, the intake of drugs such as those leading to gingival enlargement, systemic diseases such as leukemia, immune deficiencies, and diabetes mellitus, and other conditions such as stress [77]. Recent evidence also suggests that genetic polymorphisms such as those in the IL-1 gene cluster may contribute to susceptibility to gingivitis [81]. The clinical significance of these factors is that patients who have them (eg, pregnant women or children born with Down syndrome) are more susceptible to gingivitis and are therefore at greater risk for disease. Consequently, these patients require more stringent plaque control practices. Other factors such as smoking and anti-inflammatory drugs lead to a muted response [77], which results in gingiva appearing healthier than expected based on the amount of plaque present. This muted gingival response in smokers could mask severe underlying disease (ie, periodontal attachment loss) unless the clinician performs a complete periodontal examination that includes periodontal probing.

Periodontitis

Ample evidence suggests that susceptibility to periodontitis varies considerably among individuals, with approximately 10% being highly susceptible and 10% being highly resistant [9]. This difference in susceptibility has largely been attributed to genetic factors [82,83]. In a study of adult twins, it was estimated that 50% of the risk for chronic periodontitis is accounted for by heredity [83]. The specific genes that might be implicated in chronic periodontitis have not been determined. A specific genetic marker (IL-1 genotype) has been associated with greater susceptibility to periodontitis in some populations and with poorer

long-term prognosis (ie, greater tooth loss) following treatment [84,85]. Similar to the findings for chronic periodontitis, genetically determined susceptibility has also been demonstrated for LAP, although no specific gene mutations have been identified to date [86]. Although there are no reliable means at present to predict susceptibility to periodontitis, evidence suggests that susceptibility to periodontitis may be linked to susceptibility to gingivitis [77].

Genetic diseases associated with periodontitis, such as Down syndrome [87] and Papillon-Lefèvre syndrome [88], highlight the importance of hereditary factors in determining susceptibility to periodontal disease. Furthermore, when the primary genetic defect and the ensuing biochemical and physiologic implications are known, such diseases can provide significant insights regarding pathogenesis. The recent identification of specific gene defects underlying systemic diseases whose phenotype includes periodontitis—cyclic neutropenia [89,90], severe congenital neutropenia [91,92], Chédiak-Higashi syndrome [93–95], Papillon-Lefèvre syndrome [88,96,97], leukocyte adhesion deficiency [98,99], and Cohen syndrome [100,101]—has enhanced the understanding of how molecules and cells can be implicated in periodontal destruction (Table 3). Cyclic neutropenia, severe congenital neutropenia, Cohen syndrome, and Chédiak-Higashi syndrome represent various forms of quantitative neutrophil defects, underscoring the protective function of neutrophils (polymorphonuclear leukocytes; PMNs) against infections (see later discussion). Immunologic abnormalities common to Chédiak-Higashi and Papillon-Lefèvre syndrome include abnormalities in natural killer cells (see later discussion). Leukocyte adhesion deficiency is characterized by PMNs that cannot migrate to the site of injury or infection; this defect results in absence of extravascular PMNs despite increased PMN numbers in the circulation. It should be noted that in addition to those already described, other specific immunologic and genetic defects might be associated with the aforementioned diseases. An individual's genetic background, which cannot be modified, is not the only factor that can influence disease susceptibility. Several acquired or

Table 3
Genetic diseases associated with periodontitis

Genetic disease	Implicated gene	Affected protein	Year identified [reference]
Leukocyte adhesion deficiency	*ITGB2*	Integrin, beta-2	1986 [99]
Chédiak-Higashi syndrome	*LYST*	Lysosomal trafficking regulator	1996 [95]
Papillon-Lefèvre syndrome	*CTSC*	Cathepsin C	1999 [88,97]
Cyclic neutropenia	*ELA2*	Neutrophil elastase	1999 [90]
Severe congenital neutropenia	*ELA2*	Neutrophil elastase	2000 [92]
Cohen syndrome	*COH1*	COH1	2003 [101]

environmental factors, most of them modifiable, have been strongly implicated (see the article by Albandar within this issue for further exploration of this topic). The two most significant risk factors are smoking and diabetes mellitus. Smoking, in a dose-dependent manner [102], greatly increases the risk for chronic periodontitis and generalized aggressive periodontitis [103–106]. Smokers, compared with nonsmokers, have deeper probing depths, more attachment loss, greater bone loss, more rapid disease progression, and more tooth loss; in addition, they respond less well to periodontal therapy and are more prone to lose teeth during maintenance [106]. The various mechanisms through which smoking increases susceptibility to periodontitis include negative effects on PMN functions, on humoral and cellular immune responses, on fibroblast function, and on the vascular bed, among others [106]. The only positive aspect of the strong relation of smoking to periodontitis susceptibility is the fact that smoking is a modifiable factor; smoking cessation has been shown to be of significant benefit in reducing the risk of disease activity [105,106]. There are several treatment modalities available to the clinician to help his or her patients achieve a smoke-free lifestyle [106,107], therefore decreasing their susceptibility to periodontitis.

Diabetes is reaching epidemic proportions in the United States and elsewhere [108,109], and persons with diabetes are twice as likely to have periodontitis as nondiabetics [104,110]. Diabetes increases susceptibility to periodontitis and other infectious diseases through several mechanisms. One essential mechanism appears to be the nonenzymatic glycation of proteins and lipids, resulting in the formation of advanced glycation end products (AGEs) [111,112]. AGEs appear to preferentially alter the functionality of the target cells of diabetes (eg, endothelial cells and monocytes) through specific cell surface receptors such as the receptor for AGEs. When the AGE-induced activation of cell surface receptor for AGEs is experimentally blocked in diabetic animals, periodontitis-associated alveolar bone loss is decreased [111]. The altered functionality of endothelial and monocytic cells results in vascular changes and in exacerbated inflammatory responses; the inflammatory responses include increased levels of inflammatory cytokines such as TNF-α and matrix-degrading enzymes such as collagenase [111,112].

Host cells and molecules implicated in periodontal pathogenesis

The accumulated dental bacterial plaque has the potential to cause periodontal tissue damage directly, through mechanisms such as matrix-degrading enzymes and molecules that impair the functions of host cells as described previously. It appears, however, that plaque elicits most periodontal tissue injury through indirect mechanisms dependent on initiation and propagation of inflammatory host tissue reactions. The classic studies of Page and Schroeder [113] provided the basic understanding of gingivitis and periodontitis histopathology and inflammation. The

inflammatory infiltrate of periodontitis is characterized by PMNs, macrophages, lymphocytes, plasma cells, and substantial loss of collagen. This inflammatory infiltrate has cellular elements associated with acute (PMN) and chronic (lymphocytes, plasma cells) reactions. There are complex interactions between these host defense cells and the periodontal structural elements. The major cellular structural elements of the periodontium are the epithelial cells, the periodontal ligament and gingival fibroblasts, and the cells of the alveolar bone (eg, osteoblasts and osteoclasts). Molecular structural elements are the extracellular matrix components such as the various collagens and the noncollagenous proteins (eg, elastin and connective tissue proteoglycans). The nature of the interactions between these components determines whether a typical response to plaque accumulation results in marginal clinical inflammation (gingivitis) alone or in irreversible destruction of the attachment apparatus (periodontitis). The following paragraphs review the role of specific host cells and molecules, focusing on recent findings.

Neutrophils

The primary purpose of an inflammatory reaction, be it acute or chronic, is to contain or eliminate the injury-causing agent (eg, bacterial lipopolysaccharide) and to initiate the cascade of events that will result in repair of any tissue damage. In this regard, PMNs are the first line of defense against bacteria, and proper PMN functionality is essential for protecting the integrity of the periodontium [114]. In most patients with LAP and other conditions associated with periodontitis, PMNs have been shown to exhibit defects in chemotaxis or phagocytosis [114]. The strong association of periodontitis with genetic diseases characterized by quantitative (eg, cyclic neutropenia) or qualitative (eg, leukocyte adhesion deficiency syndrome) PMN defects provides convincing evidence of the critical protective role of PMNs. PMNs and other host components essential for defense against microorganisms, however, may also be central participants in the tissue destruction seen in periodontitis and other infectious/inflammatory diseases [115]. For example, activated PMNs have been shown to cause damage to gingival epithelial cells [116] and periodontal ligament fibroblasts [117]. The significance of the PMNs in this aspect of periodontal disease pathogenesis is also supported by other findings. Increased tissue levels of PMNs have been associated with active (destructive) periodontal lesions [118], whereas salivary or gingival crevicular fluid levels of neutrophil proteolytic enzymes such as collagenase and elastase correlate with disease activity or clinical indices of disease [119,120].

Elastase (a neutrophil enzyme that has indirect antibacterial properties) and collagenase can degrade several components of the extracellular matrix (eg, various collagens and elastin), thus destroying the three-dimensional scaffolding necessary for tissue organization. The significance of collagenase

in periodontitis tissue damage has been clinically exploited by the introduction of nonantimicrobial-dose doxycycline therapy [121–123]; low-dose doxycycline inhibits host collagenase without having any discernible effect on the microflora present [121,123]. In addition to the aforementioned PMN proteolytic enzymes, the neutrophil may contribute to tissue destruction through the release of reactive (toxic) oxygen metabolites [116,124]. These reactive oxygen-containing molecules can also degrade periodontal matrix molecules directly [125] and indirectly through activation of latent enzymes [126] or inactivation of enzyme inhibitors [127].

Recent evidence from LAP studies suggests that a hyper-responsive or "hyperfunctional" PMN, as opposed to a "hypofunctional" or "deficient" one, may cause enhanced tissue damage in this disease [128]. PMN hyper-responsiveness, which may manifest as increased levels of intracellular signaling molecules [129] and reactive oxygen metabolites [130], appears to be genetic in origin.

Macrophages

Macrophages are mononuclear cells responsible for orchestrating an immune response and that participate in the early, nonspecific, or innate defense against microorganisms and in specific immunity through their antigen-presenting function. Macrophages can modulate these responses through the various cytokines they produce. Studies in the last decade have shown that macrophages present in periodontitis lesions have varied phenotypes or subsets [131], suggesting diverse functionality. Unlike what has been shown for PMNs [118], there is no evidence of significant changes in the number of macrophages when tissues from healthy, gingivitis, and periodontitis sites are compared [132,133]. Furthermore, there is little evidence of macrophage activation in periodontitis lesions [133]. The significance of the various macrophage subsets to the disease process remains to be elucidated.

Natural killer cells

Natural killer cells are a lymphocyte subset involved in the innate immune response. Natural killer cells play a vital role in host defenses against infected and malignant cells by recognizing and killing such cells and by producing cytokines such as TNF-α and interferon-γ that in turn help regulate other immune cells. Natural killer cell levels increase significantly from healthy human gingiva to diseased periodontal tissues [134,135], suggesting their involvement in the immune response to plaque accumulation. Impaired natural killer cell functionality has been recently described in various systemic conditions associated with periodontitis (eg, Papillon-Lefèvre syndrome [136], Chédiak-Higashi syndrome [137], and smoking [138]), suggesting that natural killer cells serve a protective function in the periodontium.

T lymphocytes

T cells, one of two major lymphocyte subsets, are mononuclear cells whose activation is essential for cell-mediated immunity. There are several types of T cells, which are categorized by their surface antigens and functional properties; the two most common are helper and cytotoxic T cells. Helper ($CD4^+$) T cells, the target of HIV infection, function primarily by proliferating and activating (''helping'') other lymphocytes such as B cells and other T cells that ultimately are directly involved in the specific immune response against a pathogen or antigen. Cytotoxic ($CD8^+$) T cells, as their name implies, function as destroyers of target cells (typically infected or cancerous cells) that express specific antigens on their surface. $CD4^+$ and $CD8^+$ T cells are present in periodontitis lesions; their ratio and relative numbers are thought to reflect the regulatory status of the local immune response [139,140]. One of the most exciting recent findings about T cells and their contribution to periodontal pathogenesis involves the understanding of their direct involvement in periodontal bone resorption [141,142]. This understanding stems from the discovery of osteoprotegerin ligand, a molecule that is critical for osteoclast formation and lymphocyte and lymph node development and regulation. T cells activated by periodontopathogens produce osteoprotegerin ligand, which directly stimulates the formation of osteoclasts through its binding to receptor activator of nuclear factor kappa B (RANK). RANK is a cell surface receptor molecule that is highly expressed on osteoclast precursors and osteoclasts and is critical for osteoclast differentiation and activation. In addition to osteoprotegerin ligand and RANK, a third related molecule is involved in bone homeostasis: osteoprotegerin. Osteoprotegerin, produced by osteoblasts and other cells, is a decoy receptor that circulates and binds osteoprotegerin ligand and thus prevents osteoprotegerin ligand from activating RANK [143]. Osteoprotegerin ligand and osteoprotegerin are produced by periodontal cells, and their ratio is altered, favoring osteoprotegerin ligand, in diseased periodontal tissues [144–146]. Exogenous osteoprotegerin administration can inhibit bacteria-induced alveolar bone resorption [141] and represents a promising future treatment modality to prevent periodontal and peri-implant bone loss.

B cells

B cells, the second major lymphocyte subset, are essential for humoral immunity. A B cell, preprogrammed to recognize a specific antigen, gives rise to plasma cells that produce specific antibodies when triggered by the antigen and other regulatory cells. The numbers of B cells increase from health to gingivitis to periodontitis [113,147], as does the ratio of B cells to T cells [132]. The presence of significantly higher B-cell levels in active periodontitis lesions suggests that B-cell activation contributes to disease progression [148].

Proinflammatory cytokines and lipid mediators

The vast array of signaling molecules participating in the complex cellular interactions taking place in the tissue can be categorized as proinflammatory and anti-inflammatory (Table 4). In many instances, it is the balance between these two types of signals that determines the tissue response and the initiation or progression of disease. The following proinflammatory mediators are some of the best studied in relation to periodontal disease pathogenesis.

Interleukin-1. The contributions of IL-1α and IL-1β (two distinct but related molecules, collectively referred to as IL-1 here) to alveolar bone loss and periodontal disease have received considerable attention [149]. IL-1, produced by monocytic, epithelial, osteoblastic, and other cells, is a potent stimulator of bone resorption and inhibitor of bone formation. Several periodontopathogens can stimulate IL-1 production by host cells (see the Lipopolysaccharide section above), and IL-1 levels are elevated in diseased periodontal tissues. In humans, local levels of IL-1 increase in gingivitis [150] and in periodontitis [151], whereas periodontal therapy significantly decreases such levels [151]. In recent nonhuman primate experiments, use of a specific IL-1 inhibitor resulted in significant reduction of periodontopathogen-induced attachment loss, bone resorption, and inflammation [152]. These results suggest that IL-1 inhibitors might be useful in the management of periodontitis.

Tumor necrosis factor-α. TNF-α is a molecularly distinct cytokine that shares many biologic activities with IL-1. TNF-α has been implicated in the periodontal disease process because of its ability to stimulate bone resorption and other catabolic processes. The use of TNF inhibitors (in combination with IL-1 inhibitors) results in decreased inflammation and tissue destruction in nonhuman primates [153,154]. Recent clinical studies and complementary evidence from animal experiments suggest that TNF-α may be of particular significance in the exacerbated periodontal tissue destruction associated with diabetes [111,155,156].

Prostaglandins and thromboxanes. Prostaglandin E_2 and thromboxane B_2 are lipid molecules produced by many host cells through the cyclooxygenase pathway, one of the two major paths of arachidonic acid metabolism. Among other studies, the use of flurbiprofen (a nonsteroidal anti-inflammatory drug that inhibits prostaglandin E_2 and thromboxane B_2

Table 4
Host molecules regulating the inflammatory response

Molecules	Proinflammatory	Anti-inflammatory
Cytokines	IL-1α, IL-1β, TNF-α	IL-10
Lipid molecules	Prostaglandin E_2, thromboxane B_2	Lipoxin A_4

production) helped demonstrate that these mediators contribute to the redness and bleeding of gingivitis [157] and the alveolar bone loss of periodontitis [158].

Anti-inflammatory cytokines and lipid mediators

The discovery of host-derived anti-inflammatory molecules is a much more recent development compared with the isolation of their proinflammatory counterparts.

Interleukin-10. IL-10 is a cytokine with potent anti-inflammatory properties that regulates humoral and cellular immune responses and the production of several proinflammatory cytokines such as IL-1 and TNF. Recent animal studies demonstrate that lack of IL-10 leads to significantly greater alveolar bone loss [159,160], whereas recent clinical evidence indicates that gingival tissue IL-10 levels are significantly greater in healthy compared with periodontitis sites [161,162]. These and other results suggest that IL-10 might be of use as biologic therapy in periodontitis.

Lipoxins. Lipoxins are produced through the lipoxygenase pathway, the second major path of arachidonic acid metabolism. Recent evidence suggests that lipoxin A_4, in addition to other molecules in this class, can modulate the host response to promote resolution of inflammation, in general, and in response to periodontopathogens, in particular [163,164]. This evidence raises the possibility of active anti-inflammatory therapy with lipoxins (or analogs thereof) as means to control the inflammation-induced tissue damage in periodontitis.

Summary

The last decade or so has brought significant advances to the understanding of periodontal disease pathogenesis. These advances include the discovery of new, uncultivated, disease-associated bacterial species; the recognition of dental plaque as a biofilm; the identification and characterization of genetic defects that predispose to periodontitis; the role of risk factors in disease susceptibility; and the discovery of new host-derived cellular and molecular mechanisms implicated in periodontal tissue destruction. Many of these discoveries hold promise for the future as foundation for the engineering of new prevention and treatment modalities.

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