

Chapter 4

Research Advances in Periodontal Etiopathology

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Introduction

Periodontal diseases are amongst the most common inflammatory diseases of humans. They are characterized by bacteria-induced inflammatory destruction of tooth-supporting tissues. Gingivitis is clinically characterized by gingival redness, edema, bleeding, change in counter, loss of tissue adaptation to tooth surface, and increased flow of gingival crevicular fluid (GCF) (American Academy of Periodontology, AAP 1999). It may remain contained to the marginal gingival tissues or it may develop into periodontitis with nonreversible destruction of periodontal attachment and alveolar bone, which eventually results in tooth loss. In general, gingivitis precedes periodontitis, but not all gingivitis develops into periodontitis (Lindhe *et al* 1973, Listgarten *et al* 1985, Löe *et al* 1986). A number of epidemiological studies have shown that while periodontal diseases are prevalent in the population, advanced loss of tooth-supporting tissues only affects limited amounts (usually less 20%) of the population in both developed and developing countries, despite dental plaque being a common finding in a majority of the population (Löe *et al* 1986, Holmgren *et al* 1994, Söder *et al* 1994, Baelum *et al* 1996, Papapanou 1996). Studies on the nature of progression of periodontitis have

revealed that the overall pattern of progression is episodic and infrequent with active and quiescent phases, rather than the previously assumed linear fashion. For the majority of periodontitis subjects, most of the diseased sites are relatively stable at any given time, and only a small proportion exhibit active disease progression (Lindhe *et al* 1983, Socransky *et al* 1984). It is conceivable therefore that active periodontitis could occur more frequently and affect multiple sites in susceptible individuals.

Existing evidence in periodontal pathogenesis has demonstrated that periodontal diseases are initiated and perpetuated by a group of predominantly gram-negative and anaerobic bacteria that colonize the subgingival environment. It has become apparent that although bacteria are essential, they are insufficient by themselves for periodontitis to occur and for the disease outcome. Instead, the severity of periodontal diseases is dependent upon a dynamic equilibrium of interactions between microbial challenge and host immuno-inflammatory response. These events are significantly influenced by genetic, environmental or acquired disease modifiers (Page *et al* 1997). This notion is fundamentally important to further understanding the concepts of the etiology, pathogenesis, prevention, diagnosis and treatment of periodontal diseases.

Over the past two decades, substantial new

findings have been obtained regarding the etiopathogenesis of periodontal diseases. The emergence of novel findings and concepts can be well reflected in the recognition of dental plaque as a biofilm; identification and characterization of periodontopathogens including novel microbes and bacterial virulence factors in subgingival biofilm; appreciation of the importance of host-microbe symbiosis and interactions in periodontal health and diseases; molecular mechanisms of periodontal destruction; and the impacts of various risk factors on bacteria-host interactions. This article briefly reviews the current findings and novel concepts regarding the etiology and pathogenesis of periodontal diseases.

Dental bacterial plaque as a biofilm

It has been proven that dental plaque exists in the form of a microbial biofilm (Marsh and Bradshaw 1995), which is defined as “matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces” (Costerton *et al* 1995). With respect to the molecular organization, physiochemical properties and growth characteristics, biofilms are considered etiological communities that evolved to permit survival of the community as a whole and to allow species growth, enhanced virulence and evasion of host defense mechanisms. The structure of a biofilm is characterized by varying areas of high and low bacterial biomass interlaced with aqueous channels of different size (Costerton *et al* 1995, Costerton and Lewandowski 1997) which provide nutrients for the bacterial colony. GCF is the main nutritional component found in the ecosystem accounting for the predominance of asaccharolytic species (Darveau *et al* 1997). Some of the crucial properties of biofilm bacteria which have been identified and characterized include cell to cell communication through quorum sensing or so called cell density-mediated gene expression, gene

transfer, regulation of gene expression, and antimicrobial resistance (Tatakis and Kumar 2005). Of these, the property of antimicrobial resistance allows bacterial cells in the biofilm to develop and enhance resistance to host antimicrobial mechanisms like phagocytosis and antimicrobial agents, which should be taken into account in clinical practice. In this regard, periodontitis is one of the most unusual opportunistic infections where an oral biofilm forms on non-shedding tooth surfaces which could render the host defenses and antimicrobial therapy ineffective (Socransky and Haffajee 1997).

Periodontopathogens and bacterial virulence factors

The primary role of bacteria in the etiology of most forms of inflammatory periodontal diseases has been well established (AAP 1996). Over 500 different species have been identified in subgingival biofilm, but of these only approximately 20-30 species are considered to be of pathogenic significance in periodontal diseases. It is believed that only a limited number of these species play a major pathogenic role in the disease (Dahlén 1993). The transition from gingival health to gingivitis and then periodontitis is associated with an increased total number of subgingival gram-negative anaerobic bacterial species (Van Winkelhoff 1998). Specific microorganisms associated with periodontal health, gingivitis and various forms of periodontitis have been extensively reviewed recently (Tatakis and Kumar 2005) and it seems that different periodontal diseases have somewhat unique profiles of associated bacteria. However, the characteristics of microbiological progression from periodontal health to gingivitis, and eventually to periodontitis are vast and complicated (Moore *et al* 1982). Seminal studies on cluster analysis of subgingival plaque biofilm by Socransky and Haffajee and their

colleagues (1998) have shown that certain specific species frequently occur together in groups or complexes with a color-coded identity. These represent bacterial consortia that are associated with the microbiological transformation from 'beneficial biofilms' in periodontal health, characterized by predominantly gram-positive, aerobic and non-motile microflora, to 'pathogenic biofilm' in periodontitis, characterized by a gram-negative, anaerobic and motile microflora. For details, the readers are referred to Socransky *et al* (1998), Holt and Ebersole (2005) and Socransky and Haffajee (2005). The 'red complex' comprises the three major periodontopathogens, i.e. *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* (previously *Bacteroides forsythus* or *Tannerella forsythensis*). It has been suggested that the 'red complex' presents as a portion of the climax community in the subgingival biofilms which are strongly associated with progressing periodontitis (Socransky *et al* 1998, Holt and Ebersole 2005).

Most of the periodontopathogens concerned exhibit particular virulence factors (Darveau *et al* 1997, Ishikawa *et al* 1997). In the past decade, the study of potential virulence factors produced by periodontal pathogens is a very active area of research. Most of the studies that have investigated virulence factors of known or presumed periodontal pathogens have examined factors produced by *P. gingivalis* (Haffajee and Socransky 2005). The readers should refer to Holt and Ebersole (2005) and Tatakis and Kumar (2005) for more detailed reviews of virulence factors of periodontopathogens.

In general, the expression of virulence is related to various factors including the nature of the microbes, infection patterns, characteristics of the environmental niche and the host responses to the microbial complexes in the biofilm. The common specific virulence

factors associated with periodontopathogens consist of lipopolysaccharide (LPS), heat shock proteins, extracellular proteolytic enzymes, fimbriae, outer membrane proteins, leukotoxin, flagellum, and capsule. Of these, LPS has been intensively studied. It is produced by most gram-negative bacteria, such as *P. gingivalis*, *T. denticola* and *T. forsythia*, and it is a primary inducer of chronic inflammatory diseases and septic shock. LPS is also the best-characterized pathogen-associated molecular pattern (PAMP) able to trigger host inflammatory response by inducing the release of inflammatory mediators and cytokines, such as interleukin (IL)-1 α , IL-1 β , tumor necrosis factor- α (TNF- α), IL-8, and prostaglandin E₂, through CD14 and toll-like receptor-mediated activation of neutrophils, macrophages and fibroblasts. The other effects on host cells deal with induction of nitric oxide secretion, activation of osteoclasts and stimulation of T-helper cell proliferation. It has been shown that unlike other bacteria, *P. gingivalis* LPS does not initiate an immediate innate host inflammatory response and in fact suppresses the innate inflammatory response to bacteria by inhibiting E-selectin expression, which may represent a new virulence factor for this organism (Darveau *et al* 1995, Darveau *et al* 1997, Reife *et al* 1995). Heat shock protein 60 (hsp60) has been increasingly recognized as a crucial molecule in infectious and autoimmune diseases. It enabled stimulatory activity similar to LPS derived from the bacteria. A recent study has shown that it was abundantly expressed in periodontitis lesions and it has therefore been postulated that periodontopathogens stimulate the cells in the periodontium to up-regulate the expression of hsp60, which in turn may stimulate macrophage and possibly other cells to produce pro-inflammatory cytokines, which could contribute to the chronicity and tissue destruction of periodontal disease (Ueki *et al* 2002). It has been shown that the hsp from *Actinobacillus*

actinomycetemcomitans could activate osteoclasts and stimulate epithelial proliferation (Paju *et al* 2000). Traditionally, potential virulence factors produced by pathogens were studied using bacterial cells that were grown *in vitro*. It has recently been realized that a number of pathogens express virulence genes only when they are in their human or animal host (Haffajee and Socransky 2005). An unique and ingenious approach to detecting and distinguishing such virulence factors as well as examples of its use in analyzing virulence factors for periodontal disease has recently been described by Handfield and coworkers (2005).

Novel microbes in subgingival biofilm

It is estimated that at least 10^{14} normal or commensal microbes reside on the surfaces of skin, teeth, dentures, dental restorations, prosthetic implants, as well as the mucosal epithelia lining of oral cavity, respiratory, gastrointestinal, and urinary tracts (Cohen and Slavkin 2000). The oral cavity contains approximately six billion microbes representing 500-700 species (Socransky and Haffajee 1994, Wilson *et al* 1997, Paster *et al* 2001). Up to 300 oral bacterial species can be cultured from oral plaque samples, yet it is estimated that another 300 species are uncultivated. Under certain conditions these commensal organisms could become opportunistic pathogens contributing to local and/or systemic infections. Recent evidence has increasingly shown that oral opportunistic pathogens and their resultant oral infections, for instance periodontitis, have been implicated in a number of systemic diseases or disorders, e.g. endocarditis, coronary heart disease, cerebral infarction (or stroke), diabetes, pre-term birth, and aspiration pneumonia (Williams and Offenbacher 2000, Jin *et al* 2003). Our recent studies showed that microbes normally inhabiting non-oral niches could be found from

dental plaque specimens of systemically compromised subjects (Leung *et al* 1998, Leung *et al* 2003). Knowing this, the human oral microbial ecosystem would therefore be highly relevant for the diagnosis and treatment of oral opportunistic infections and related systemic diseases (Paster *et al* 2001).

Environmental surveys based on acquisition of phylogenetically useful microbial sequences, such as that of the 16S rRNA gene, have revealed a great deal of previously unsuspected bacterial and archaeal diversity. In most instances, cultivated members represent <1% of the total extant population (Kroes *et al* 1999). In recent years, the human subgingival crevice has been the focus for intensive studies, as cultivation methods woefully under-represent the true extent of microbial diversity. The current best approach for exploring unidentified species is based on isolating DNA from the target environment, PCR amplifying the rRNA gene, cloning the amplicons into *E. coli*, and sequencing the cloned 16S rRNA gene inserts (Paster *et al* 2001). Recently, a number of novel oral phylotypes (e.g. uncultivated division of TM7) have been identified from periodontitis specimens by molecular phylogenetic methods, such as methanogenic *Archaea*, *Desulfobulbus* sp oral clone R004, *Deferribacteres* sp oral clones BH017 and D084, and *Bacteroides* sp oral clone AU126 (Kroes *et al* 1999, Dewhirst *et al* 2000, Kulik *et al* 2001, Paster *et al* 2001, Kumar *et al* 2003, Lepp *et al* 2004).

Moreover, viruses have also been considered potential members of the oral microbial ecosystem. Human cytomegalovirus, herpes simplex virus and Epstein-Barr virus have been detected in subgingival plaque by nested- or real-time-PCR assay and they were associated with the severity of periodontal disease (Slots *et al* 2003, Kubar *et al* 2005). In general, herpes virus infection can increase the incidence of bacterial and fungal infections, aggravate the severity of concurrent microbial

infections, and accelerate the *tempo* of infectious disease progression (Boeckh and Nichols 2003). Viruses and bacterial pathogens may also act synergistically in oral infectious diseases and further study of these is warranted.

Host-microbe symbiosis and interactions in periodontal health and diseases

The indigenous microbes of humans consist of a number of microbial communities, each with a composition characteristic of a particular body site. It has been estimated that there are ten times more bacteria colonizing a human than the number of human cells in the body, i.e. 10^4 versus 10^3 (Wilson 2005). In the past decade, great progress has been made in further understanding of human-microbe symbiosis (Wilson 2005). In the past, investigations of the bacterial-host interaction focused upon pathogenesis. Currently, it is believed that representative interactions are not pathological but symbiotic. In fact, both humans and bacteria benefit from peaceful coexistence and microbial colonization plays an obligatory role in human health (Tuomanen 2005).

In the context of periodontology, it has recently been proposed that bacteria and their products are a necessary and beneficial component of a healthy periodontium, with the evidence that clinically healthy periodontal tissue contains a highly orchestrated gradient of select inflammatory mediators. These play a key role in the defense of periodontal tissues and the overall health of the individual and these mediators are made in response to a highly specific microbial consortium residing on the tooth surface (Roberts and Darveau 2002). It is conceivable that balanced and appropriate interactions of beneficial microbes and periodontal tissues contribute to a healthy and functional periodontium. Two conceptual advances in the field of immunology have

provided a framework to understand this issue, i.e. the human immune system has evolved to recognize threats (Matzinger 1998) and the theory of pattern recognition originally proposed by Janeway (1992) to explain how the innate host defense system evolved a mechanism to recognize microbes immediately and mount a protective response. The latter proposes that the innate defense system recognizes common conserved structures of different microbes, such as LPS, rather than the specific microbes; and a group of designated pattern recognition receptors, such as lipopolysaccharide binding protein (LBP), CD14, and the Toll-like receptor family (TLR), continually monitor the state of host microbial colonization and elicit appropriate host responses depending upon the microbial structures detected. Pattern recognition receptors therefore provide a link facilitating a molecular dialogue between the commensal bacteria and the appropriate host response (Roberts and Darveau 2002).

It has become increasingly clear that the innate immune system has a much more important and fundamental role in host defense (Medzhitov 2000). Innate host responses are initiated by a variety of microbial PAMPs like LPS and subsequently modulated by LBP, CD14, TLR superfamily (e.g. IL-1RI, TLRs-2 and 4) and MD-2 that recognize various PAMPs and subsequently initiate the transduction of transmembrane signaling cascades through mediation of adaptive proteins, such as MyD88, MyD88-adaptor-like (Mal), IRAK family, TRAF-6 and downstream signaling, leading to the activation of nuclear factor- κ B (NF- κ B) and eventually induction of cytokine gene expression (Aderem and Ulevitch 2000). It has been appreciated that LBP, sCD14 and BPI play important roles in facilitation of neutralizing and clearance of LPS by cells through formation of LPS-LBP-sCD14 complex or BPI-LPS aggregates without leading to cellular activation (Tapping and Tobias 1997, Weiss 2003).

Pattern recognition molecules and antimicrobial peptides in periodontal health and disease – Our recent work

Our recent *in vivo* and *in vitro* studies have focused upon the identification and characterization of common pattern recognition molecules that could be crucially important for periodontal health. We firstly detected sCD14 in GCF and found its levels were significantly higher than those in serum, indicating that this pattern recognition receptor could be produced locally in response to microbial challenge. Furthermore, higher levels of sCD14 in GCF were associated with fewer and shallower periodontal pockets (Jin and Darveau 2001). This study shows that sCD14 may serve a protective role in local host response to bacterial challenge. A descriptions of the expression profile of mCD14 in gingival tissues was undertaken. mCD14 protein and mRNA were commonly detected in healthy or diseased gingival tissues. The mCD14-positive cells were mainly confined to the gingival epithelium-connective tissue interface. Expression levels in periodontally healthy subjects were significantly higher than in the patients. Within the patients, clinically healthy tissues showed greater levels of mCD14 than periodontal pocket tissues and granulation tissues. These findings suggest an important role of mCD14 in the mediation of effective immunoinflammatory responses to bacterial challenge (Jin *et al* 2004). LBP is an acute-phase reactant, predominantly derived from the liver. It may serve to both neutralize LPS and enhance its biological activities of cellular activation. We recently discovered that LBP protein and mRNA can be locally expressed in gingival epithelia and its expression was mainly confined to the cytoplasm of granular and keratinized layers of gingival epithelium, spreading from the oral sulcular epithelium to oral epithelium with the expression density decreasing gradually from coronal to apical

portion (Ren *et al* 2004). Furthermore, it was also observed that LBP mRNA was more frequently expressed in healthy tissues than in diseased tissues and the expression levels of LBP protein were higher in periodontally healthy tissues than in diseased tissues. It could be speculated that local expression of LBP in gingival tissues might contribute to periodontal homeostasis. Moreover, our recent *in vitro* study revealed that recombinant human LBP (rhLBP) could significantly down-regulate the expression of both mRNAs and peptides of IL-6 in the presence or absence of *E. coli* LPS, and suppress the up-regulated expression of TLR-2 and -4 by *E. coli* LPS (Ren *et al* 2005a). Further studies are warranted to clarify the molecular mechanisms of LBP in regulation of cytokine expression by host cells and to elaborate the relevant clinical implications. Our most recent study further explored the potential interrelationship of *in vivo* expression of LBP and mCD14 in human gingival tissues as well as the co-expression of TLR-2 and -4 in association with periodontal health and disease (Ren *et al* 2005b). A positive correlation was found between LBP and mCD14 peptides in both detection expression and expression levels of these relevant molecules. In diseased tissues, TLR-2 was detected in both pocket epithelia and macrophage-like cells in connective tissues; while TLR-4 was predominantly detected in connective tissues. However in healthy tissues, only a weak expression of TLR-2 was found in gingival epithelia and no TLR-4 expression was detected. In periodontal pocket tissues, mCD14 was co-detected on CD68-labelled macrophages in the underlying connective tissues of pocket epithelium as well as on CD1a-labelled dendritic cells in the pocket epithelium and connective tissues interface. No similar expression profile was detected in healthy tissues from patients and those from periodontally healthy control subjects. These novel findings on an altered cellular expression profile of mCD14 and TLR-2 and -4 in

periodontal pocket tissues imply that these pattern recognition receptors may play a crucial role in periodontal pathogenesis.

It is now well recognized that gingival epithelia serve not merely as physical barriers to microbial challenges, but rather as reservoirs of antimicrobial peptides which enable them to survive under normal as well as harsh environmental conditions. Of the various attributes contributing to innate immunity, a group of well-evolved and conserved antimicrobial peptides, human α - and β -defensins, which are detectable in gingival epithelia, are now considered to be of major importance in innate host defense (Dale 2002). The expression pattern of α - and β -defensins has been described that α -defensins are usually located in the junctional epithelium produced by neutrophils, while β -defensins are distributed in sulcular and oral epithelia, suggesting defensins serve different roles in various regions of the periodontium (Dale *et al* 2001). We recently further described the expression patterns of human β -defensins (hBD) 1-3 in both periodontal health and disease (Lu *et al* 2004, Lu *et al* 2005). The expression of both hBDs-1 and -2 peptides was mainly confined to the granular and spinous layers of gingival epithelium, while hBD-3 peptides were mainly detected in the basal layer in health and the expression extended from the basal layer to the spinous layers in diseased condition. hBD-3 peptide was expressed not only in gingival keratinocytes but also in Langerhans cells and Merkel cells. Furthermore, periodontally healthy tissues expressed higher levels of hBD-2 peptides than clinically healthy tissues from periodontitis patients. These data suggest that appropriate expression of hBDs-1 and -2 may contribute to maintenance of periodontal homeostasis, while hBD-3 peptides may contribute to the maintenance of periodontal homeostasis, possibly through its antimicrobial effect and promotion of adaptive immune responses.

Taken together, our data suggest that an appropriate expression and regulation of host pattern recognition receptors (LBP, sCD14, mCD14, TRL-2 and -4) and local antimicrobial constituents (hBDs) are crucial for maintenance of periodontal homeostasis, despite the relevant regulation mechanisms and intracellular signaling pathways are not fully understood.

Molecular mechanisms in periodontal destruction

Basic principles of infectious diseases indicate that disease expression is a combination of host, microbial agents and environment factors. In the light of the new paradigm of periodontal pathogenesis, it is believed that bacterial flora is necessary but not sufficient for expression of periodontal diseases and there are many other host response factors and environmental/genetic factors which dramatically modify the disease outcome (Page *et al* 1997). The current paradigm of periodontal pathogenesis has shifted and this places renewed emphasis on the host response factors. The complex interplay between the bacterial challenge and innate and acquired host response factors determines the disease outcomes, i.e. the conversion of junctional epithelium to pocket epithelium, formation of periodontal pocket, destruction of periodontal attachment, and alveolar bone loss. It is known that bacterial biofilm can directly cause periodontal injury and that bacteria elicit the most periodontal destruction through indirect mechanisms such as initiation and up-regulation of host destructive inflammation, especially in a periodontally susceptible individual (Page *et al* 1997). With an ongoing microbial challenge, the periodontium is continuously exposed to virulent bacterial components which could alter local cell functions and phenotypes. The host defense cells which are significantly involved are

neutrophils, macrophages, lymphocytes and plasma cells. Meanwhile, complex interactions exist between the defense cells and structural/resident cells including epithelial cells, fibroblasts, osteoblasts and osteoclasts, as well as structural components including various collagens and non-collagenous proteins, through up-regulated cytokines and inflammatory mediators like PGE₂. The total impact of the above alterations is to shift the host response from one in which the host could contain the bacterial challenge to one in which the infection is no longer under control while destructive inflammation is predominant (Kornman *et al* 1997a). The dynamic events of periodontal pathogenesis are determined primarily by the signaling and regulating molecules (e.g. cytokines and prostaglandins) that direct cellular functions. It has been proposed that active periodontitis is characterized by high levels of IL-1 β , TNF- α , INF- α , PGE₂ and MMPs, and low levels of IL-10, TGF- β , IL-1ra and TIMPs that suppress the immuno-inflammatory response and maintain homeostasis (Page *et al* 1997). It is realized that under these pathological conditions, defense cells and structural cells such as fibroblasts are activated in an uncontrolled manner and they produce large amounts of MMPs and pro-inflammatory cytokines and mediators, whilst decreasing the production of TIMPs, resulting in tissue destruction. Subsequently, pro-inflammatory cytokines and PGE₂ mediate hyperactivity of osteoclasts whilst suppressing the osteoblast activity, leading to the resorption of alveolar bone.

Genetic defects and host susceptibility

As mentioned above, although bacteria are essential, they are insufficient for disease to occur nor directly responsible to the severity of the disease, while a susceptible host is necessary for determination of the severity of the disease. It is believed that the disease

severity is rather dependent upon a dynamic equilibrium of bacteria-host interactions which are significantly influenced by various genetic and environmental factors (Page *et al* 1997, Kinane *et al* 2005). In this regard, periodontal disease is obviously a multi-factorial complex disease. It is well evident that severe forms of periodontal disease affect a minority of the subjects (Løe *et al* 1986). A range of risk factors which have been studied include subject determinants, social and behavioral factors, systemic factors, genetic factors, tooth factors and microbial risk factors (Nunn 2003). Emerging evidence also shows that periodontal infection *per se* may be a potential risk factor for systemic diseases like cardiovascular disease (Jin *et al* 2003).

The first convincing demonstration that not everybody is at equal risk to periodontal destruction from periodontitis, despite inadequate oral hygiene and hence exposure to microbial dental plaque, came from an Asian study. Løe and co-workers identified three subgroups within the studied subjects on the basis of periodontal destruction, which were labelled "no progression", "moderate progression" and "rapid progression" subgroups (Løe *et al* 1986). It is now appreciated that the existence and combinations of various factors may significantly account for an individual's risk for periodontal destruction. The current risk factors include subject characteristics; social and behavioral factors, such as tobacco smoking, socio-economic status, nutrition and psychological factors; systemic factors, such as diabetes mellitus, drugs, HIV; genetic factors considering genotype polymorphisms; tooth-level factors, such as anomalies and poor restorations and the microbial composition of dental plaque featuring the holy triumvirate of *A. actinomycetemcomitans*, *T. forsythensis* and *P. gingivalis* (Nunn 2003); and other emerging risk factors, such as obesity (Saito *et al* 2001). Of these, tobacco smoking is believed to be

the most important environmental risk factor for periodontitis (Kinane and Chestnutt 2000).

In recent years, genetic factors as a crucial determining risk for periodontitis have received considerable attention among periodontal researchers. For details, the readers are referred to an excellent recent review by Kinane *et al* (2005). Genetic defects may significantly predispose an individual to severe periodontitis. The current genetic factors include defects of phagocytosis resulting in a hypo-response to the bacterial challenge, or enhanced production of cytokines, prostaglandins, and MMPs, in response to bacterial challenge. There is clearly a fine balance in the nature of the inflammatory response to the presence of plaque, and both under-activity (hypo-responsiveness) and over-activity (hyper-responsiveness) of components of the host response can result in increased susceptibility to disease and destruction of periodontal tissues (Preshaw *et al* 2004). It has been shown that variations in genotypes of inflammatory cytokines and mediators may be independent risk factors for severe periodontitis, such as IL-1 (Kornman *et al* 1997b, Kornman and di Giovine 1998) and other cytokine polymorphisms (e.g. IL-6, IL-10 and TNF), receptor polymorphisms (e.g. RcyRIIa, VDR-TaqI) and other host response polymorphisms (e.g. HLA-DQ β , MMP-1-2G). We recently found that a single nucleotide polymorphism in the MMP-1 promoter region of -1607 bp may be associated with generalized aggressive periodontitis in a Chinese population (Cao *et al* 2005).

Of the gene polymorphisms above, the IL-1 genotype has been intensively investigated. Some studies show that the subjects with the IL-1 composite genotypes of allele 2 of both IL-1 α (+4845) and IL-1 β (+3954) produce more IL-1 than the controls and are at a relatively high risk for developing severe periodontitis than those individuals without the target genotype. Other studies in different

populations have questioned the general usefulness of this test in clinical practice in various ethnic populations. It has been reported that the prevalence of the IL-1 composite genotype was lower in Chinese (2.3%), suggesting the usefulness of IL-1 composite genotype for determining susceptibility in Chinese subjects is dubious (Armitage *et al* 2000). The current concerns for genetic testing in periodontal research seem to focus on assessment of multiple gene polymorphisms, such as IL-1 α , TNF- α , IL-6 (D'Auito *et al* 2004), Comprehensive 125 Genes 310 SNPs (Suzuki *et al* 2004), and TNF- β (NcoI bi), ACE (I/D), Endothelin-1 (TaqI) (Holla *et al* 2001).

Clinical implications and perspectives

Our updated knowledge and concepts of periodontal etiopathogenesis enhance the main targets of conventional periodontal treatment for effective disruption of bacterial biofilm and control of plaque retention factors, through mechanical instrumentation by non-surgical approaches with periodontal surgery as necessary. It has been shown that mechanical instrumentation is by far the most effective approach to control plaque biofilm and it will continue to be the cornerstone of periodontal therapy. Research advances in the pathogenesis of periodontitis and appreciation of the crucial role of the host response have significantly contributed to the newer treatment strategies, i.e. emphasizing the identification of high risk individuals through risk assessment and controlling risk factors in clinical management of periodontitis, and development of adjunctive host modulatory therapies to modulate destructive components of the host response for better periodontal treatment outcomes. The new research advances in etiology and pathogenesis of periodontal disease hold promise for development of novel strategies for preventing and controlling periodontal disease in humans.

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