The Role of Inflammatory Cytokines and Interleukin-6 Expression /Gene Polymorphism in Chronic Periodontitis: Review of the Literature

Nikolaos Andreas Chrysanthakopoulos*

*Corresponding author:
Dr. Nikolaos Andreas Chrysanthakopoulos, University of Athens, 35, Zaimi Street, PC 26 223, Patra, Greece, Tel./Fax: 0030-2610-225288; E-mail: nikolaos_c@hotmail.com, nchrysant@med.uoa.gr

Abstract

Periodontitis is a chronic inflammatory condition of the periodontal tissues involving interactions between bacterial products, inflammatory mediators, and various cell populations. It is accepted that periodontitis is initiated by the dental plaque formation on the teeth surfaces. Dental plaque ingredients such as antigens, lipopolysaccharides, and other factors, initiate an inflammatory and immune response, leading to the activation of host cells which results in the release of inflammatory mediators such as cytokines, chemokines, arachidonic acid metabolites and proteolytic enzymes which contribute to tissue destruction and bone resorption. Recent research regarding the pathogenesis of periodontal diseases has clarified the involvement of various cytokines in the biological activities that are implicated in its pathogenesis. Cytokines play crucial roles in the maintenance of tissue homeostasis, whereas excessive or continuous cytokines production in inflammatory periodontal tissues is responsible for the progress of periodontitis and periodontal tissue destruction. Inflammatory cytokines-such as IL-6, IL-13, and IL-8 are present in the damaged periodontal tissues, and their production is involved in chronic leukocyte recruitment, tissue and bone destruction. It is possible that cytokine production and expression may result to diagnose an individual's periodontal disease status or susceptibility to the disease. Moreover, despite the contradictory suggestions, it has been proposed that T-cell subsets Th1 and Th2 with different cytokine profiles play crucial roles in the immunopathogenesis of periodontal disease. Chronic periodontitis is suggested to be related to gene variations whereas; genetic variations which are observed in cytokines may affect periodontitis susceptibility. The aim of the current review was to present the role of inflammatory cytokines and Interleukin-6 expression and its gene polymorphism in chronic periodontitis pathogenesis.
Keywords: Interleukin-6, single nucleotide polymorphism, chromic periodontitis, genotype

Introduction

Periodontal Disease (PD) affects nearly 50.0% of adults in the United States, whereas 8.5% of those have been diagnosed with severe periodontitis. In addition, PD is regarded as one of the most common diseases worldwide, showing a prevalence of 10%-15% (1). Risk of PD initiation and progression is associated with smoking history, diabetes mellitus, age, poor oral hygiene, furcation involvement, residual pocket depths, frequency of supportive periodontal care and genetics (2-6).

Periodontal pathogens that have been shown to be responsible for the PD initiation and progression, are mainly Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia, and are considered the most important pathogens in periodontitis (7,8). Periopathogen products initiate an inflammatory reaction that leads to local tissue destruction including connective tissue and bone support loss, which may eventually result in tooth loss (9). The toxic products of the subgingival periopathogens disrupt the immune surveillance and enhance the toxic environment of the periopathogens leading to a disruptive tissue homeostasis which concerns connective tissue, periodontal ligament, cementum and alveolar bone (10). The inflammation progression induces the production of insistent inflammatory signals by the cells of periodontal tissues. Such signals, which involve the proinflammatory cytokines, IL-1, IL-2, and Tumor Necrosis Factor-alpha (TNF-α) induce the production of secondary inflammatory mediators which in turn amplify the inflammatory signalling pathways, that are able to lead to an increased production of proteases and osteoclastic signals that result in connective tissue and bone destruction (11,12).

Chronic Periodontitis (CP) is a chronic inflammatory pathological condition caused by the interaction between microorganisms that produce supragingival and subgingival biofilms and the host inflammatory response (13), and is characterized by the inflammatory gingiva and the destruction of tooth-supporting structures (14). CP etiopathogenesis involves many factors such as environmental and genetic factors (15-18). The development of CP is promoted by the interaction between periodontal pathogens and the host immune response (13,19). Host immune re-sponse of the periodontal tissues is caused by bacterial infection, whereas, the inflammatory response of the periodontal tissues is influenced by environmental and genetic factors (15,20). Concerning the genetic factors, cytokine genes may play an important role in the immune pathology of PD and their analysis of polymorphisms may be possibly associated with it (21). Those gene polymorphisms may cause an alteration in the encoded cytokine, or its expression, possibly resulting in alterations in innate and adaptive immunity, and may hence be a definitive factor of PD outcome determination (22,23).

One of the most investigated cytokines genes whose polymorphisms are associated with PD development is IL-6. That cytokine shows various cellular functions, including differentiation and activation of macrophages and T-lymphocytes and development and final differentiation of B-lymphocytes. IL-6 levels regulation plays a crucial anti-inflammatory role in both systemic and local inflammatory reactions (24). Previous reports have shown associations between IL-6 gene polymorphisms and periodontitis in different ethnicities and it has been proposed that IL-6 -174G/C polymorphism is associated with periopathogens
identification, variations in inflammation severity, attachment loss and tissue destruction in PD (25,26). Individual changes IL-6 blood levels may modify the predisposition to many inflammatory diseases, such as PD, as it is a proinflammatory mediator that activates host cells and may lead to extracellular matrix destruction. Such IL-6 changes are regulated by common nucleotide variations in its encoding gene, which is located on chromosome 7p21 (27).

Pathogenesis of Periodontitis

Periodontitis has been considered to be a complex interaction between the bacterial invasion and the host response, as already mentioned, which alters connective tissue and bone metabolism and causes periodontal damage, as clinical attachment loss (28). PD consists of two main pathological conditions, gingivitis and periodontitis and is a progressive condition, which leads to supporting tissue destruction and in case of periodontitis leads to alveolar bone loss. Severe periodontitis and edentulism influence 743 and 158 million individuals worldwide, respectively (29,30). Gingivitis, is characterized by inflammation of the gingival tissue, while in susceptible individuals, gingivitis may progress to periodontitis (23).

Although periopathogens are responsible for the PD initiation, the host immune response is implicated in its pathogenesis as it seems to play a crucial role in the destruction of connective tissue and bone. The mentioned immune and inflammatory reaction differs among individuals due to individuals genetic factors as their susceptibility, environmental factors and possible polymorphisms in cytokines and chemokines that are implicated in those reactions. Bacterial antigens and toxic agents, such as lipopolysaccharides (LPS), peptidoglycans, lipoteichoic acids, proteases and toxins released by bacteria, are recognized by toll like receptors (TLRs) on the surface of host cells, induce an immune and inflammatory reaction, in which innate and adaptive immune systems are involved (31,32). The host response to the bacterial invasion includes the stimulation of various inflammatory cell types such as polymorphonuclear leukocytes (PMN), monocytes, macrophages, B- and T-lymphocytes (28,33). The inflammatory reaction activates mast cells which in turn release vasoactive amines, TNF-α, and various inflammatory mediators that are able to increase vascular permeability and the expression of adhesion molecules such as inter-cellular adhesion molecule-1 (ICAM-1) and P-selectin on endothelial cell surfaces. PMNs are recruited into the damaged tissue, and release lysosomal enzymes, such as glycosidases, proteases, sulfatases which contribute to connective tissue breakdown (34). Despite the fact that macro-phages and lymphocytes contribute to the destruction of a rate of 60-70% of the collagen in the gingival connective tissue, no alveolar bone resorption is observed at that phase (35). The gingival tissues damage at this phase is still reversible and it is possible to be repaired and restored after removal of the etiological factors. However, in some cases the inflammatory reaction fails to resolve and macrophages form preosteoclasts which, after maturing into osteoclasts are responsible for the irreversible alveolar bone loss, due to innate susceptibility and/or environmental factors (36). Another implication of an unsolved inflammatory reaction is that the bacterial antigens interact with antigen presenting cells (APC) such as dendritic cells, macro-phages and B-lymphocytes. The interaction between naive CD4 (+) T helper cells (T0) with APC, leads to the differentiation of those into various subsets of cells including Th1, Th2, Th17 and regulatory T cells (Tregs), and this differentiation depends on the
cytokines that they produce, such as IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, IL-22, IL-23, TNF-α, TGF-β, IFN-γ, etc. Th1 cells are responsible for initiation of the cell-mediated immune response and release IFN-γ, TGF-β, IL-2, and TNF-α in the presence of IL-12, which is released by dendritic cells, macrophages and PMNs cells. Th2 cells are responsible for the humoral immune response and release the cytokines IL-4, IL-5, IL-6, IL-10, IL-13 and TGF-β in the presence of IL-4. Th17 subset of cells produce IL-17, -23, -22, -6 and TNF-α in the presence of TGF-β, IL-1β and -6, whereas Tregs appear in the presence of TGF-β and release the immunosuppressive cytokines IL-10 and TGF-β. IL-17 is implicated in the pro-duction of various inflammatory mediators such as TNF-α, PGE2, IL-6 and -1β, mediating bone resorption by osteoclasts activation (34,36). Tregs and Th17 cells are present in periodontal tissue with an increased expression of Foxp3 and IL-17, suggesting their crucial role in the immunoregulation of periodontitis (33,34,37). It has also been shown a plasticity between Th17 and Treg cell subsets which are present in the same tissues, and especially in periodontitis lesions (37). However, the role of the association between the Treg/Th17 and Th1/Th2, and their cross-talk in the pathogenesis of periodontitis it still remains unclear.

The inflammatory reaction involves in addition to recruited inflammatory cells, blood vessels, and their endothelial cells and smooth muscle cells that are come in contact with the recruited inflammatory cells. Gingival fibroblasts, resident cells of the connective tissue, produce inflammatory mediators, such as cytokines, chemokines, proteolytic enzymes and PGs which are involved in the inflammatory response and contribute to disease persistence (38-44). Periodontal ligament fibroblasts, which are located between the tooth and the alveolar bone, are also implicated in the inflammatory reaction and produce inflammatory mediators such as PGs, proteolytic enzymes and factors which affect bone resorption. During the inflammatory process, proinflammatory mediators are released and in turn affect various cell types and promote the inflammatory pathway (45-47).

Cytokines and Chemokines

Various pro-inflammatory cytokines such as IL-1, -6, -12, -17, -18, -21, TNF-α and IFN-γ and chemokines such as IL-8, Monocyte Chemoattractant Protein-1 (MCP-1), Macrophage Inflammatory Protein-1α (MIP1α), and chemokine (C-C motif) ligand 5 (CCL5) have been identified in the Gingival Crevicular Fluid (GCF), and in gingival tissue from patients with periodontitis and are implicated in its pathogenesis (48).

TNF-α is released from mast cells in response to bacterial antigens and has a crucial role at an early phase of the inflammatory reaction. Periodontal tissue cells that also produce TNF-α and IL-1 are PMN cells, monocytes/macrophages, epithelial and endothelial cells, fibroblasts, and osteoblasts (48). Those inflammatory cytokines are increased in GCF and gingival tissue with periodontitis (49-51). Their role in periodontitis has been proven as after periodontal treatment their levels were reduced (52, 53). Experimental studies have shown that soluble receptors of IL-1 and TNF-α inhibit the progress of periodontitis in a primate model (54,55). During the inflammatory reaction those cytokines are implicated
in the induction of other inflammatory mediators, such as IL-6, -8, Matrix Metalloproteinases (MMPs) and PGE2, whereas are also involved in bone resorption (56-60).

IL-6 is a pro-inflammatory cytokine, released by lymphocytes, monocytes, epithelial cells, and fibroblasts in response to bacterial LPS, IL-1 and TNF-α and is implicated in the formation of osteoclasts in vitro (57,61). Periodontitis patients’ GCF and gingival tissue are characterized by increased levels of IL-6, compared with healthy locations (62, 63). Similarly, after nonsurgical periodontal treatment IL-6 levels have been found to be decreased (64). Chemokines are cytokines implicated in inducing chemotaxis in responsive cells. IL-8 is a chemokine released by various cells, such as epithelial and endothelial cells, monocytes, lymphocytes, and fibroblasts, in response to IL-1, TNF-α and LPS and recruits neutrophils and other leucocytes to the inflammation site (56, 65). In aggressive periodontitis gingival tissues show increased levels of IL-8 expression, which especially are identified to areas with high concentrations of PMN cells (66). Similarly, elevated levels of IL-8 have been found in GCF in periodontitis sites compared with healthy ones, whereas its levels were decreased after periodontal treatment (67).

The chemokine MCP-1 is produced by epithelial cells, fibroblasts, and endothelial cells, in response to bacterial endotoxins such as LPS or inflammatory mediators (57, 68), whereas in periodontitis MCP-1 and MIP-1α attract neutrophils and other leucocytes to the inflammation site (56,65). In periodontitis pathogenesis is also involved the chemokine (C-C motif) ligand 5(CCL5) as they have been found elevated levels in gingival biopsies and/or GCF, whereas after periodontal treatment were identified decreased levels of CCL5 and other chemokines (49,53,69-73).

IL-1α, -1b, -12 (p40), -17, -18, -21, IFN-γ and TNF-α cytokines have been found to be increased in GCF in periodontitis patients(49,52,53, 62,69-71,73-79). Similarly, chemokines such as MCP-1, MIP1a, and CCL5 were also increased in periodontitis patients (53,70,74).

**Interleukin-6 Expression and Gene Polymorphism**

Bacterial invasion and environmental factors are implicated in initiation and modulation of PD progression, however, evidence supports that genes have an important role in the predisposition to and progression of periodontitis (80,81).

Cytokines and oral pathogens play central roles in the inflammatory process associated with the etiology of periodontitis (82-84). Polymorphisms in the regulatory regions of the cytokine genes may change cytokine expression. The cytokine gene polymorphism and expression could be contribute to the identification of genetic factors that play a significant role in the periodontitis etiology and define the susceptibility of individuals with respect to PD and clinical outcomes (85).

Based on previous reports it seems that IL-6 is implicated in the periodontitis pathogenesis (23,86). The development of periodontitis is characterized by biological events that could be mediated by the binding of IL-6 and its receptor (IL-6R), including immunocyte activation, angiogene-sis, hematopoiesis, and
osteoclast differentiation (87). IL-6 levels are elevated in periodontitis patients (88,89) and decreased after successful periodontal therapy (90,91), whereas in those patients IL-6 has been identified in serum, GCF, and salivary, condition that proposes a modified production of IL-6 (92,93). IL-6 release has been associated with the persistence of oral inflammation and tissue destruction caused by proteases, osteoclasts, and methylation alterations (94-97), whereas increased levels of IL-6 expression is positively correlated with attachment loss (98). IL-6 is also a potent stimulator of osteoclast differentiation and bone resorption (99,100), whereas also regulates the expression of cytokines such as IL-1, -10, and TNF-α (101,102).

As already mentioned genetic factors may predispose to periodontitis (80,81). Thus, several studies have been carried out to research whether IL-6 gene polymorphisms predispose to periodontitis. However, the results regarding the possible associations between these polymorphisms and clinical forms of periodontitis are conflicting.

Clinical studies indicated that IL-6 plays a crucial role in the inflammatory response to Gram-negative bacteria (103) by affecting the constitution of the subgingival microbiota and increasing the susceptibility to colonization with periopathogens bacteria, and in general is induced in response to several inflammatory stimuli (104-107).

The simple nucleotide polymorphism (SNP) of the IL-6 promoter gene may affect the production and expression of that cytokine, and consequently, this change in serum levels may result in a relevant biological response (108).

Previous studies have reported an association of the IL-6-174G/C polymorphism and periodontitis and suggested that IL-6 may be considered an important marker for its pathogenesis (4,109-112). Nibali et al. (25) suggested a positive association between IL-6 -174 G/G genotype and the presence of A. actinomycetemcomitans and Capnocytophaga sputigena in subgingival plaque. Similarly, Shao et al. (113) in a systematic review and meta-analysis demonstrated that IL-6 G/G genotype could not modify the risk of CP, but increased the risk of aggressive periodontitis. An increased expression of IL-6 and IL-6 -174G/C polymorphism was found to be associated with PD severity in Brazilian individuals (98), whereas in another study was found that IL-6 -174 G/G genotypes and G allele seems to be associated with aggressive periodontitis (114) and the extend of it (115). Trindade et al. (26) reported associations between cytokine gene polymorphisms and periodontitis in distinct populations. The effect of that polymorphism on the susceptibility of CP has been studied by multiple researchers and has been found positive associations (110,116-121). The IL-6 -572 C/G polymorphism has also been studied in the context of susceptibility to periodontitis. However, compared with C allele carriers (genotypes GC and CC), the GG genotype significantly increased the risk of CP, suggesting that the -572 G allele is associated with the pathogenesis of CP under the compliant genetic model (122-125). It has also been shown that the same polymorphism was associated with the pathogenesis of periodontitis, as it predisposes to either CP or aggressive periodontitis (113).
Polymorphisms in the regulatory regions of genes may alter the expression of cytokines revealing an important role of genetic predictors of disease susceptibility and clinical measures (126). More specifically, IL-6 -572 G/C polymorphisms have been associated with an increased protein expression and inflammatory response (127).

Besides the IL-6 -174 G/C and -572 C/G polymorphisms, the -6331 T/C polymorphism in the promoter region of the gene could also alter the transcriptional activity of IL-6. It is suggested that IL-6 -6331 T/C polymorphism is implicated in the pathogenesis of periodontitis, but that association with risk of periodontitis requires to be confirmed in other ethnics (128).

A study by Nibali et al. (129) supported the hypothesis of a link between IL-6 genetic factors and aggressive periodontitis and highlights the importance of two IL-6 polymorphisms (-1363 and –1480) in modulating disease phenotype and susceptibility. Another similar study (125) suggested the hypothesis that IL-6 polymorphisms and haplotypes are moderately associated with periodontitis, possibly acting through influencing tissue levels of IL-6.

On the contrary, some reports have shown no association between the polymorphism IL-6 -174 G/C and PD. Fan et al. (130) observed a very low prevalence of the C allele and found no significant difference in the genotypes distribution for the IL-6-174G/C polymorphism in the analysis of groups with PD and coronary artery disease. Similarly, Holla et al. (122) found no significant differences between patients with periodontitis and healthy controls regarding the genotype or allele frequencies between both groups for IL-6 -174 G/C and-597 G/A polymorphisms. Another study conducted by Wohlfahrt et al. (131) also revealed no association between IL-6 -174G/C polymorphism and other polymorphisms in CTLA-4, DEFB1, ICAM-1, FasL, ICOS, CCR5, OPG, and OPN genes in individuals with PD. The same study showed no significant association between this polymorphism and CP, finding that was in accordance with the results of a previous analysis (132). Loo et al. (133) showed no association between genetic polymorphisms in IL-6,-1b and IFN-γ genes and PD however, in the same study, associations with polymorphisms in IL-1, TNF-α, IL-4 and -10 genes were described. Ianni et al. (134) found no association, whereas in another report (130) was found that IL-6 -572C/G polymorphism did not correlate with CP susceptibility, but might be a potential risk factor for Coronary Heart Disease in a Chinese population. In addition, Holla et al.(122) suggested that the -572 G/C polymorphism of the IL-6 gene may be one of the protective factors associated with lower susceptibility to chronic periodontitis. It was also observed that the IL-6-373 A9T11 allele was associated with a reduced susceptibility to CP among Japanese individuals and decreased IL-6 serum levels (124).

It is clear that the prevalence of IL-6 polymorphism varies in different samples and populations, and it might also vary the association with susceptibility to periodontitis. That observation would lead to discrepancies and contradictory results in genetic polymorphism studies carried out on periodontitis patients of different ethnicities and various genetic backgrounds. Except from racial and genetic differences, other differences could be attributed to possible confounders such as clinical diagnosis criteria, environmental variables, biologic plausibility, and significant heterogeneity among the studies reviewed, penetrance and logic of association studies (135).
References


11. Costa AM, Guimaraes MCM, de Souza ER, et al. Interleukin-6(G-174C) and tumour necrosis factor-alpha (G-308A) gene polymorphisms in geriatric patients with chronic periodontitis. Gerodontology 2010;27:70-75.


68. Preshaw PM, Taylor JJ. How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? J Clin Periodontol 2011;38:60-84.


