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Expression, polymorphism and methylation pattern of interleukin-6 in periodontal tissues

Florença Abdanur Stefani[a], Michelle Beatriz Viana[a], Ana Carolina Dupim[a], João Artur Ricieri Brito[a], Ricardo Santiago Gomez[a], José Eustáquio da Costa[b], Paula Rocha Moreira[c,*]

[a] Laboratory of Molecular Biology, Department of Oral Surgery and Pathology, School of Dentistry, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[b] Department of Periodontology, School of Dentistry, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[c] Laboratory of Cell-Cell Interactions, Department of Morphology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

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ABSTRACT

Periodontitis is considered an inflammatory disorder of bacterial etiology that results in periodontal tissue destruction, as a result of complex interactions between periodontal pathogens, host and immune response. Genetic and epigenetic mechanisms may modulate the individual response since it is able to influence the gene expression. The aim of this study was to evaluate the impact of −174 G/C polymorphism and the methylation status of the promoter region of *IL*-6 gene on the expression of IL-6 in gingival samples from individuals with chronic periodontitis. Gingival biopsies were collected from 21 patients with chronic periodontitis and 21 controls. Histologic sections stained by hematoxylin–eosin were used for histopathological evaluation. The IL-6 gene expression was assessed by quantitative real-time PCR. The polymorphism IL-6 −174 C/G was studied by polymerase chain reaction (PCR) amplification and restriction endonuclease digestion (*HspII*). Methylation-specific polymerase chain reaction was used to verify the DNA methylation pattern. The number of inflammatory cells in tissue fragments from individuals with chronic periodontitis was higher than in the control group and the inflammatory infiltrate was predominantly mononuclear. The expression of IL-6 was higher in the group with periodontitis. In polymorphism assay, no statistical difference in the distribution of genotypes and alleles in both groups were observed. The most of samples were partially methylated. No difference was observed in methylation pattern from two different regions of the IL-6 gene among groups. The high expression of IL-6 is an important factor related to chronic periodontitis, but was not associated with methylation status or the −174 (G/C) genetic polymorphism, suggesting that other mechanisms are involved in this gene transcription regulation.

Introduction

Periodontitis is considered an inflammatory disorder initiated by the presence of Gram-negative bacteria which results in periodontal tissue destruction (Kinane and Hart 2003; Shapira et al. 2005). Periodontitis outcomes are influenced by immune and inflammatory response to bacterial challenge, as well the environmental factors (Page and Kornman 1997). It has been related the presence of an individual susceptibility to development of periodontal disease (Kornman et al. 1997; Albandar 2002; Shao et al. 2009). Individuals presenting similar clinical aspects may respond differently to the same periodontal therapy, which highlights the importance of the host factors in pathogenesis of periodontal diseases.

The host response involves complex interaction between cells, extra cellular matrix and circulating cytokines (Irwin and Myrillas 1998). Cytokines play a fundamental role in the immune response and may to lead to a protective immunity or the tissue destruction by the production of different profiles of cytokines (Gemmell and Seymour 2004). Cytokines interact in a network and the balance among local levels of these mediators is important in determining the outcome of an immune response (Gemmell et al. 1997). Among several kinds of cytokines involved in periodontal disease, the interleukin-6 (IL-6) has been shown increased in the gingival crevicular fluids and in the gingival tissues of individuals with periodontitis (Takahashi et al. 1994; Armitage 1996; Offenbacher

Abbreviations: Bp, base pair; CAL, clinical attachment loss; cDNA, complementary deoxyribonucleic acid; DNA, deoxyribonucleic acid; IFN-γ, interferon-gamma; IL-6, interleukin 6; MSP, methylation-specific polymerase chain reaction; PCR, polymerase chain reaction; PD, probing depth; qPCR, real-time polymerase chain reaction; RNA, ribonucleic acid; UFMG, Universidade Federal de Minas Gerais.

* Corresponding author at: Laboratório de Biologia das Interações Celulares, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Avenida Antônio Carlos 6627, Pampulha, CEP 31.270-901, Belo Horizonte, Minas Gerais, Brazil.
E-mail address: paularocha@ufmg.br (P.R. Moreira).

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1996). The IL-6 is considered an inhibitor of bone formation and plays an important role in development of disease (Sodek and McKee 2000; Irwin et al. 2002; Mercado et al. 2003).

It has been reported that IL-6 expression is influenced by genetic alterations and also by multiple epigenetic events, such as DNA methylation and histone modification. The −174 G/C single nucleotide polymorphism has been reported to influence the IL-6 expression, with the G allele associated to high expression levels (Fishman et al. 1998). Recently, studies have associated the −174 G/C *IL-6* polymorphism with the susceptibility and severity to chronic periodontitis (Trevilatto et al. 2003; Moreira et al. 2007).

Epigenetic is described as changes in pattern of gene expression, which do not involve changes in the nucleotide sequence (Egger et al. 2004; Feinberg 2008). Alterations in the DNA methylation status have been implicated in cancer and inflammatory process (Armenante et al. 1999; Kishi et al. 2005; Nile et al. 2008; Mi and Zeng 2008; Gomez et al. 2009). Current investigations suggests that epigenetics mechanisms are able to influence the cytokine production and, consequently, to alter the clinical features of inflammatory diseases (Fitzpatrick and Wilson 2003; Addock et al. 2002; Gomez et al. 2009).

Considering the multifactorial nature of periodontal disease, it is possible to consider that genetic and epigenetic events could contribute to the development and phenotype of disease (Schekein 2002; Offenbacher et al. 2008). Since IL-6 has a crucial role in the pathogenesis of periodontal disease, the comprehension of the mechanisms able to alter its expression may be important to understand the development of the inflammatory process. Therefore, the purpose of the present study was to evaluate the impact of −174 G/C polymorphisms and the methylation status of the promoter region of *IL*-6 gene on the expression of IL-6 in chronic periodontitis.

Materials and methods

Sample collection

The present study was approved by the Research Ethics Committee from the Universidade Federal de Minas Gerais – UFMG (ETIC-337/08) and a signed informed consent of all subjects was obtained. The individuals were informed about the purpose and methods of study.

A total of 42 samples of gingival tissue from individuals under treatment at the Dentistry School, Universidade Federal de Minas Gerais, and at the Centro de Especialidades Odontológicas da Prefeitura Municipal de Contagem were included in this study. All subjects were submitted to anamnesis and to clinical, periodontal, and radiographic examination. The samples were categorized according to the classification of American Academy of Periodontology (Armitage 1999) into two groups: periodontitis group ($n = 21$) and control group ($n = 21$). The periodontitis group was composed by samples of gingival tissues from individuals diagnosed with chronic periodontitis. The fragments were obtained from sites with probing depth (PD) ≥ 4 mm, with gingival bleeding and clinical attachment loss (CAL) ≥ 3 mm (López et al. 2002). The sites were classified in relation to severity; sites exhibiting PD < 7 mm were considered with moderate and those exhibiting PD ≥ 7 mm were considered as severe disease (Trevilatto et al. 2003). The control group was composed by samples of gingival tissues from individuals with absence of signs of periodontal disease. The fragments were obtained from sites with PD < 4 mm. The gingival biopsies of the periodontitis group were obtained during periodontal surgical therapy and those of the control group were obtained during surgery for non-disease-related reasons, such as dental extraction for orthodontic treatment or of third molar and crown-lengthening procedures. The fragments were immediately included in Tissue-tek (Sakura Finetek, CA, USA) for DNA extraction and in RNA holder (BioAgency Biotecnologia, São Paulo, Brazil) for RNA extraction and stored at −80 °C.

Table 1
Characteristics of the study group.

Characteristics	Control	Chronic periodontitis
Number of individuals (n)	21	21
Gender		
Male (%)	8 (38.1)	6 (28.6)
Female (%)	13 (61.9)	15 (71.4)
Median age (range years)	28 (18–69)	41 (25–58)

Systemic disorders that could influence the course of periodontal disease, pregnancy or lactation, chronic usage of anti-inflammatory drugs or other drug therapy, severely compromised immune function, and smokers were regarded as exclusion criteria. The characteristic of the study group are shown in Table 1.

Histological study

Two sections of 5 μm from frozen samples in Tissue tek (Sakura, CA, USA) were stained by hematoxylin–eosin for histopathological examination. The histological sections were submitted to total inflammatory cells counting and a mean number for section counted was obtained (original magnification 400×).

RNA extraction and real-time polymerase chain reaction (qPCR)

Total RNA was extracted using Trizol reagent from samples collected in RNAholder (BioAgency Biotecnologia, São Paulo, Brazil). The RNA obtained was treated with RNAse-free DNAase I (Invitrogen Life Technologies, Carlsbad, CA, USA), according to the manufacturer's protocol. The cDNA was synthetized from 1 μg of total RNA using Superscript first-strand synthesis system (Invitrogen Life Technologies, Carlsbad, CA, USA). Real-time PCR quantitative was performed using TaqMan fluorescence quantification system in a Step-One real-time PCR 48-well plate (Applied Biosystems, Warrington, UK). The reactions were performed with the TaqMan® Gene expression assay (Applied Biosystems, Warrington, UK): IL6 (Hs00985641_A1). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method as previously described (Schimittgen and Livak 2008) and the expression data were normalized with endogenous b-actin. The data were presented as the relative quantity of target gene normalized to endogenous b-actin and relative to a calibrator sample (Applied Biosystems User Bulletin No. 2 – P/N 4303859-Applied Biosystem, Foster City, CA, USA). Pool of blood samples of healthy individuals was used as calibrator.

DNA extraction

The remaining fragments of the frozen samples in Tissue tek (Sakura, CA, USA) were used for DNA extraction. The genomic DNA was extracted with the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA concentration was determined by a spectrophotometer.

Polymerase chain reaction (PCR) and restriction endonuclease digestion

IL-6 (−174 G/C) polymorphism was assessed by PCR amplification and restriction endonuclease digestion. The forward primer 5′-CAGAAGAACTCAGATGACTG-3′ and the reverse primer 5′-GTGGGGCTGATTGGAAACC-3′ were used with a product size of 431 base pairs (bp), as described previously (Klein et al. 2001).