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Investigation of an IL-2 polymorphism in patients with different levels of chronic periodontitis

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Abstract

Background: Interleukin-2 (IL-2) is a pro-inflammatory cytokine derived from Th1 cells. This cytokine is involved in B-cell activation and stimulates macrophages, natural killer cells, T-cell proliferation and osteoclast activity. IL-2 has been also implicated in the stimulation of osteoclast activity in bone resorption.

Objective: In this study the relationship between the polymorphism −330 (T→G) in the *IL-2* gene and different levels of chronic periodontal disease was investigated.

Material and Methods: DNA was extracted from buccal epithelial cells of 113 unrelated adult individuals acting as controls and with different levels of periodontitis. The PCR-RFLP technique was used to investigate the polymorphism in the promoter of *IL-2* gene.

Results: When comparing the data of three groups of patients (Control, Moderate and Severe) we did not find significant differences between the studied IL-2 polymorphism and severity levels of PD. However, when the Control and Moderate phenotypes were grouped together and compared with genotypes TT vs. TG/GG, a significant difference was observed.

Conclusion: We conclude that the −330 (T→G) polymorphism in the *IL-2* gene is associated with the severity of periodontal disease. The results presented in this study suggest an active role of IL-2 in the pathogenesis of periodontal disease.

Key words: chronic periodontitis; interleukin-2; polymorphism; risk factors

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Periodontal disease (PD) is characterized by inflammatory cell accumulation in the periodontal tissues. Since most pathogenic bacteria reside in periodontal pockets and do not invade the periodontal tissues, the immune system can never efficiently eliminate the microorganisms (Okada & Murakami 1998). This unique situation leads to a chronic inflammation and continuous host response, resulting in tissue destruction (Page 1991). The local host response to bacteria includes the recruitment of leukocytes and the subsequent release of inflammatory mediators (Okada & Murakami 1998). The involvement of cytokines in PD has been extensively studied in the past few years, but the mechanisms by which they interfere in the disease progression are not completely understood.

Gemmell & Seymour (1994) have proposed that the stable periodontitis lesion is mediated principally by cells with the Th1 cytokine profile, while the progressive periodontitis lesion involves Th2 cells. Interleukin-2 (IL-2) is a cytokine produced by T-helper 1 cells (Th1). This cytokine is involved in the B-cell activation and stimulates macrophages, natural killer cells and T-cell proliferation, which mediate the cellular immune response, being regarded as a pro-inflammatory cytokine (Tew et al. 1989, Wilson et al. 1996, Parkes et al. 1998). IL-2 has been also implicated in the stimulation of osteoclast activity in bone resorption (Ries et al. 1989). The levels of immunoreactive IL-2 in dental

pulp have been shown to be a relevant parameter to determine the extent of pulpal inflammation (Rauschenberger et al. 1997). There is evidence indicating that IL-2 may also be a relevant factor in the pathogenesis of periodontal disease. Lymphocytes cultured from the chronically inflamed periodontal tissues of patients with alveolar bone loss produced IL-2 (Seymour et al. 1985). The levels of IL-2 in the sera of periodontitis patients are elevated when compared to those of normal subjects (McFarlane & Meikle 1991). Due to its biological properties, IL-2 has been suggested to be a useful marker of pathologic inflammatory activity in systemic diseases (John et al. 1998) and periodontal conditions (McFarlane & Meikle 1991, Rauschenberger et al. 1997, Takahashi et al. 1997).

Polymorphisms in cytokine genes are associated with chronic inflammatory diseases, such as interleukin-10 (IL-10), found in high levels in systemic lupus erythematosus (Lazarus et al. 1997) and in rheumatoid arthritis patients (Perez et al. 1995). Individual differences in levels of interleukin-1 (IL-1) related to susceptibility to PD are attributed to alleles of polymorphic genes. The allele 2 of the *IL-1β* (+3953) gene is more prevalent in patients with chronic periodontitis, which indicates a connection between this polymorphism and periodontal severity in adults (Kornman et al. 1997, Gore et al. 1998). In this paper we use the term *chronic* in the place of *adult* periodontitis, according to the criteria of the *1999 World Workshop for Classification of Periodontal Diseases and Conditions* (Armitage (1999).

The investigation of polymorphisms in the *IL-2* gene concerning periodontal diseases is important because of the roles IL-2 plays in the inflammatory process. It is assumed that polymorphisms in the promoter region of genes can modify the level of expression of proteins (McGuire et al. 1994, John et al. 1998, Lazarus et al. 1997). A polymorphism in the position −330 (T→G) of the *IL-2* gene promoter was identified by John et al. (1998). The authors claim that this polymorphism could be useful as a marker to diagnose susceptibility to inflammatory diseases.

The purpose of this study was to investigate the relationship between the −330 (T→G) polymorphism in the *IL-2* gene and severity of chronic periodontal disease, to verify if such polymorphism could serve as a marker of susceptibility to this disease.

Material and Methods

Selection of subjects

A convenience sample of 113 unrelated, non-smoking subjects, >25 years of age, were recruited for study from the patient pool at the Dental Clinics of the Faculty of Dentistry of Piracicaba – UNICAMP. The patients are from the South-eastern region of Brazil. The baseline clinical parameters for the subject population are presented in Table 1. All subjects were in good general health and had at least 20 teeth. Subjects did not have any of the following exclusion criteria: diseases of the oral hard or soft tissues except caries and periodontal disease; use of orthodontic appliances; need for premedication for dental treatment; chronic usage of anti-inflammatory drugs; a history of diabetes, hepatitis or HIV infection; immunosuppressive chemotherapy; history of any disease known to severely compromise immune function; present acute necrotizing ulcerative gingivitis; current pregnancy or lactation. Subjects completed personal medical and dental history questionnaires and, within a protocol approved by an Institutional Review Board, signed a consent form after being advised of the nature of the study. Diagnosis and classification of disease severity were made on the basis of clinical parameters and consisted of physical examination, medical and dental history, probing depth, assessment of attachment loss (CAL), tooth mobility and observation of bleeding on probing. Measurements of probing depth and attachment level were recorded at six points around each tooth. Subjects were included in clinical categories according to PD generalized severity:

1 *Control group*: Subjects found to exhibit no signs of periodontal disease as determined by the absence of clinical attachment loss and no sites with probing depth >3 mm (n = 44)
2 *Moderate periodontitis*: Patients with teeth exhibiting ≥3 mm and ≤6 mm CAL (n = 31)
3 *Severe periodontitis*: Patients with teeth exhibiting ≥7 mm CAL (n = 38).

Analysis of genetic polymorphisms

Cells were obtained through a mouthwash with 3% glucose solution and light scrapings of the buccal mucosa with a sterile wood spatula. DNA was extracted from epithelial buccal cells with sequential phenol/chlorophrom solution and precipitated with salt/ethanol solution (Trevilatto & Line 2000, Scarel et al. 2000). The *IL-2* gene promoter region (accession number AJ006884) was amplified by PCR utilizing the primers: Reverse – 5′ CAT TGT GGC AGG AGT TGA GGT 3′ and Forward – 5′ TAT TCA CAT GTT CAG TGT AGT TCT 3′. The forward primer used has been described previously by John et al. (1998), with a T base in the −333 position altered to C, which creates a restriction site for the enzyme *Mae*I (C–TAG). This establishes an efficient and simple RFLP method to detect the −330 (T→G) polymorphism in the *IL-2* gene.

The PCR temperature profile included an initial denaturation step at 95 °C for 2 min, followed by 35 cycles each of 95 °C (1 min), 59 °C (1 min) and 72 °C (1 min), with a final extension step of 72

Table 1. Baseline clinical parameters of the subject population (n = 113)

	Control (n = 44)	Moderate (n = 31)	Severe (n = 38)
Age (years), mean (± SD)	43.2 (± 14.0)	36.9 (± 11.2)	43.6 (± 14.4)
Gender %			
Female	68.2	80.6	84.2
Male	31.8	19.4	15.8
Ethnic group %			
Caucasoid	84.1	77.4	68.4
Afro-American	06.8	16.1	13.2
Mulatto	06.8	06.5	18.4
Japanese	02.3	0.0	0.0

°C (5 min). RFLP was performed in a final reaction volume of 20 μL using 1.5 U *Mae*I (Boehringer-Mannheim, Indianapolis, IN, USA) and 7 μL of PCR product (410 base pair (pb)). The reactions took place overnight (ON) at 45 °C. The products were analyzed in 5% polyacrylamide gel electrophoresis. The gels were stained by the rapid silver staining method (Sanguinetti et al. 1994).

The genotype and allele frequencies were calculated by direct counting and then dividing by the number of subjects to produce genotype frequency, or by number of chromosomes to produce allele frequency (Reynard et al. 2000). The significance of the differences in observed frequencies of polymorphisms in control and diseased groups was assessed by Chi-square (χ^2) test. Differences were considered significant when $p < 0.05$. In a separate analysis we compared groups Control/Moderate vs. Severe and calculated the risk associated with individual alleles. The odds ratio (OR) with 95% confidence intervals was also used to obtain homozygous TT vs. TG/GG genotypes (Table 3). Analysis was performed with the SAS statistical package.

Results

*Mae*I enzyme digestion cleaves the PCR products in two fragments: 387 pb + 23 pb (allele 2). The results were statistically analyzed with absolute frequency (n) and percentage per column (%) of the genotypes and alleles. No significant differences among the three groups analyzed related to allelic ($\chi^2 = 5.385$, $p = 0.068$) and genotypic ($\chi^2 = 6.105$, $p = 0.191$) frequencies were found. The average frequency of allele T was homogeneous among the patients with different levels of PD (Table 2). In a separated analysis of the Caucasian subjects, no significant differences of genotypic ($\chi^2 = 3.295$, $p = 0.51$) and allelic ($\chi^2 = 2.733$, $p = 0.25$) frequencies were found among the Control, Moderate and Severe groups.

However, when we compared the Control/Moderate vs. Severe groups, the allele distribution indicated an association of allele T with the Control/Moderate group ($\chi^2 = 4.906$, $p = 0.027$) as shown in Table 3. Individuals with the T allele seem to be approximately half as likely to develop the Severe PD (OR = 1.99; 95% CI = 1.07–3.7). The frequency of genotype TT in the Control/Moderate group was also significantly different compared to the group formed by patients with severe PD ($\chi^2 = 5.479$, $p = 0.019$). Individuals with the TT genotype seem to be 2.5 times less likely to develop the severe PD than individuals who are heterozygous or GG homozygous (OR = 2.57; 95% CI = 1.15–5.73) (Table 3).

Table 2. Allele and genotype frequencies of the −330 (T→G) polymorphism in the IL-2 gene promoter in healthy patients and patients with different levels of periodontitis (n = 113)

	Control n (%)	Moderate n (%)	Severe n (%)	*p**
Allele				
T	68 (77.27)	51 (82.25)	50 (65.78)	0.068
G	20 (22.72)	11 (17.74)	26 (34.21)	
Genotype				
TT	26 (59.09)	21 (67.74)	15 (39.47)	
TG	16 (36.36)	09 (29.03)	20 (52.63)	0.191
GG	02 (04.54)	01 (03.20)	03 (07.89)	

**p* 0.05.

Table 3. Allele and genotype frequencies of the −330 (T→G) polymorphism in the IL-2 gene promoter in Control/Moderate groups vs. Severe group (n = 113)

	Control/Moderate n (%)	Severe n (%)	*p**	OR
Allele				
T	119 (77.27)	50 (65.78)	0.027	1.99 (95% CI = 1.07–3.70)
G	31 (22.72)	26 (34.21)		
Genotype				
TT	47 (62.66)	15 (39.47)		
TG	25 (33.33)	20 (52.63)	0.062	–
GG	03 (04.00)	03 (07.89)		
Genotype				
TT	47 (62.66)	15 (39.47)	0.019	2.57 (95% CI = 1.15–5.73)
TG/GG	28 (37.33)	23 (60.52)		

p 0.05.

Discussion

In spite of the pro-inflammatory roles attributed to IL-2, its involvement and influence on the progression of periodontal disease are poorly understood. High levels of IL-2 were observed in the serum of patients with periodontal pocket depths greater than 5 mm (McFarlane & Meikle 1991). However, it was found that gingival mononuclear cell (GMC) culture supernatants from inflamed tissues of chronic periodontitis patients contained no detectable IL-2 at both mRNA and protein levels (Fujihashi et al. 1993). The assessment of *in vitro* IL-2-producing capacity of peripheral blood mononuclear cells (PBMC) and lymphocytes from patients with different forms of periodontitis showed no correlation between IL-2 production and disease types (Takahashi et al. 1997). Both the healthy group and groups with periodontitis had subjects with PBMC exhibiting elevated and depressed IL-2 production. This finding may support the concept of 'high or low IL-2 producers' in response to particular stimuli and genetic factors controlling IL-2 synthesis (Molvig et al. 1988, Takahashi et al. 1997). Perhaps this idea may explain the results of McFarlane & Meikle (1991), in which many subjects may have been 'IL-2 high producers' (Takahashi et al. 1997). The expression levels of IL-2 may be modulated by genetic polymorphisms in regulatory regions of the gene. These regulatory regions exist upstream of the protein–coding region of genes and encode instructions deciphered by transcription factors, which recognize specific DNA motifs and enhance or repress the transcription (Stern 2000).

We have not found other reports in which a polymorphic marker in the promoter of the *IL-2* gene was used to determine the severity of periodontal disease in adults. When comparing the data of the three groups of patients (Control, Moderate and Severe) we did

not find significant differences between the studied IL-2 polymorphism and severity levels of PD. However, when the Control and Moderate phenotypes were grouped together and compared with the Severe group regarding genotypes TT vs. TG/GG, a significant difference was observed. This data suggest an association between the −330 (T→G) polymorphism in the *IL-2* gene and the severity of periodontal disease.

It is interesting to note that TT is the most frequent genotype of IL-2 in the population presently studied, as well as in Caucasoid individuals from the United Kingdom (John et al. 1998). It is worth mentioning that the Brazilian population is highly heterogeneous, with Native American, African and European ancestry (Alves-Silva et al. 2000). As racial differences are common in polymorphic systems (Mourant et al. 1976), it is possible that the marker used in this study may present a different correlation in the progression of periodontal disease in other populations or racial groups. In the Brazilian southern region, the European population is predominant (IBGE 1995). This is consistent with the predominance of Caucasoid individuals observed in our sample. The results presented in this study suggest an active participation of IL-2 in the pathogenesis of periodontal disease.

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Zusammenfassung

Untersuchung eines IL-2-Polymorphismus bei Patienten mit unterschiedlichen Schweregraden einer chronischen Parodontitis

Hintergründe: Interleukin-2 (IL-2) ist ein entzündungsförderndes, aus Th1-Zellen freigesetztes Zytokin. Dieses Zytokin ist an der Aktivierung der B-Zellen beteiligt und stimuliert Makrophagen, natürliche Killer-Zellen, die T-Zellen-Proliferation und die Aktivitäten von Osteoclasten. Interleukin-2 ist darüber hinaus an der Stimulierung der Osteoclasten-Aktivitäten bei der Knochenresorption beteiligt.

Zielsetzung: Ziel der vorliegenden Studie war die Untersuchung der Beziehung zwischen dem Polymorphismus –330 (T→G) im IL-2-Gen und unterschiedlichen Ausprägungsgraden chronischer parodontaler Erkrankungen.

Material und Methodik: Aus den Epithelzellen der Wangen von 113 nicht miteinander verwandten erwachsenen Freiwilligen einer Kontrollgruppe und Probanden mit unterschiedlichen Schweregraden parodontaler Erkrankungen wurde DNA entnommen. Zur Untersuchung des Polymorphismus im Promotor des IL-2-Gens wurde die PCR-RFLP-Technik herangezogen.

Ergebnisse: Beim Vergleich der Daten der drei Probandengruppen (Kontrolle, mässiger und schwerer Erkrankungsgrad) konnten wir keine signifikanten Unterschiede zwischen dem untersuchten IL-2-Polymorphismus und dem Schweregrad der parodontalen Erkrankung feststellen. Wenn die Kontrolle und die Phänotypen mit mässigem Schweregrad zusammen gruppiert und mit den Genotypen TT im Vergleich zu TG/GG verglichen wurden, konnten signifikante Unterschiede beobachtet werden.

Schlussfolgerung: Wir kommen zu dem Schluss, dass der –330 (T→G) Polymorphismus im IL-2-Gen mit dem Schweregrad der parodontalen Erkrankung in Beziehung steht. Die in dieser Studie vorgelegten Ergebnisse weisen auf eine aktive Rolle des IL-2 in der Pathogenese parodontaler Erkrankungen hin.

Résumé

Etude d'un polymorphisme de l'IL-2 chez des patients présentant divers degrés de parodontite chronique

Origine: L'interleukine-2 (IL-2) est une cytokine pro-inflammatoire dérivée des cellules Th1. Cette cytokine est impliquée dans l'activation des cellules B et stimule les macrophages, les cellules cytotoxiques naturelles (*natural killer*), la prolifération des cellules T et l'activité des ostéoclastes. L'IL-2 est également impliquée dans la stimulation de l'activité des ostéoclastes dans la résorption osseuse.

But: Cette étude a porté sur la relation entre le polymorphisme –330 (T?G) du gène de l'IL-2 et les différents degrés de maladie parodontale chronique.

Matériaux et méthodes: De l'ADN a été extrait des cellules épithéliales buccales de 113 individus adultes sans lien de parenté, comprenant des contrôles et des individus présentant différents degrés de parodontite. Le polymorphisme du promoteur du gène de l'IL-2 a été étudié au moyen de la technique PCR-RFLP.

Résultats: En comparant les données de trois groupes de patients (contrôle, modéré et grave), nous n'avons pas trouvé de différences significatives entre le polymorphisme de l'IL-2 étudié et le degré de gravité de la maladie parodontale. Cependant, en groupant les phénotypes des contrôles et des individus à parodontite chronique modérée, et en les comparant aux génotypes TT *versus* TG/GG, il existe une différence significative.

Conclusion: Nous en concluons que le polymorphisme –330 (T?G) du gène de l'IL-2 est associé à la gravité de la maladie parodontale. Les résultats présentés dans cette étude suggèrent que l'IL-2 joue un rôle actif dans la pathogénie de la maladie parodontale.

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