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Interleukin - 10 Polymorphisms in Patients with Early Onset- and Adult Periodontitis

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Introduction

Adult periodontitis (AP) and early onset periodontitis (EOP) have a bacterial etiology, but there is increasing evidence that there are different genetic and environmental factors that contribute to disease etiology and progression (Hart et al. 1997). A number of studies and case reports have demonstrated that EOP is found to aggregate within families, and investigations with twins implicate the influence of host genetic factors (Michalowicz et al. 1991). Genetic polymorphisms are known to affect both the qualitative and quantitative aspects of host response. Kornman et al. (1997) showed that polymorphisms in the IL-1 gene cluster are correlated with the severity of AP. IL-10 is a cytokine that exhibits striking activities in vitro and in vivo suggesting important functions in immune regulation. IL-10 significantly modulates expression of cytokines, soluble mediators and cell surface molecules on cells of myeloid origin. IL-10 strongly inhibits the production of several cytokines (Fig. 1) (Fiorentino et al. 1991).Genotypic variations in cytokine response have been shown in vitro for IL-10, and specific alleles are implicated in diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (Mok et al. 1998). In a recent study, 3 microsatellite marker DNA sequences of the IL-10 gene corresponding to phenotypic variations in cytokine response were analysed in patients with EOP. No links were found between EOP and the investigated markers (Kinane et al. 1999).

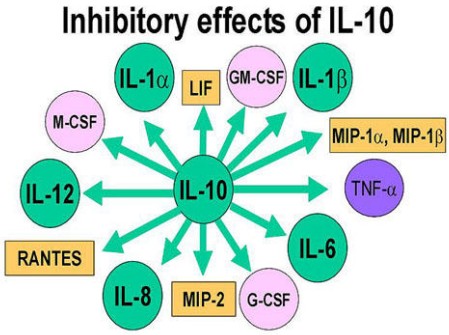

Fig. 1. IL-10 strongly inhibits the production of IL-1a, IL-1b, IL-6, IL-8, IL-10 itself, IL-12, granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colonystimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), TNF-a,m

Objective

The purpose of the present study was to investigate the influence of IL-10 promoter polymorphisms in patients with AP and EOP.

Material and Methods

23 patients with AP and 18 patients with EOP were included in the study. Additionally, 21 age-matched healthy subjects were included as a control group. Diagnosis was based on past dental history, various clinical parameters and radiographic pattern of bone loss. Periodontal diseases AP and EOP were defined as previously described by Salvi et al. (1998). For AP patients, 3 categories of bone loss were selected as described by Kornman et al. (1997). Genomic DNA was isolated fromEDTA-blood with the InstaGene® whole blood kit (Bio-Rad Laboratories GmbH, Munich, Germany).This yields typically 5 ng DNA/µl and 5-10 µl were used in the amplification reactions. The IL-10 promoter region at positions -597 and -824 was amplified by polymerase chain reaction, and polymorphisms were detected by restriction-enzyme cleavage, with slight modifications, as described by Mok et al (1998). The digested product was visualised after electrophoresis on a 3% MetaPhor®-agarose (Biozym, Oldendorf, Germany) gel stained with ethidium bromide. The *T and *C alleles at the -824 position were associated with the *A and *C alleles at the -597 position, respectively (genotype 2,2). This meant that individuals with -824*T also had -597*A, whereas those with -824*C also had -597*C.

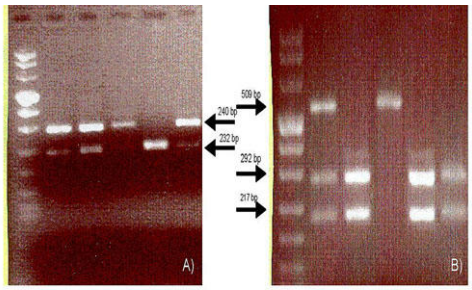

Fig. 2. Restriction pattern of IL-10. A) RsaI (position -597) gave products of 42bp + 232bp + 240bp (allele C) and 42bp + 66bp + 232 bp + 240 bp (allele A). B) MaeIII (position -824) gave products of 79bp + 217bp + 292bp (allele C) and 79bp + 509bp (allele T).

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Results

The distribution of the IL-10 genotypes according to disease category and severity is shown in Figures 3 and 4. Only one EOP (5.6%) patient and two controls (9.5%) showed the genotype 2,2. The distribution of the different genotypes 1,1, 1,2 and 2,2 in the different disease groups at both positions are shown in Figures 5 and 6 . Clinical data are presented in Figures 7, 8 and 9. As regards to bone loss, none of the AP patients had the mild stage, 17 patients showed the severe and 6 the moderate stage of periodontitis. The previously reported association between *A allele at the -597 position and the *T allele at the -824 position was confirmed in our study, although only one EOP patient and two controls carried that genotype.

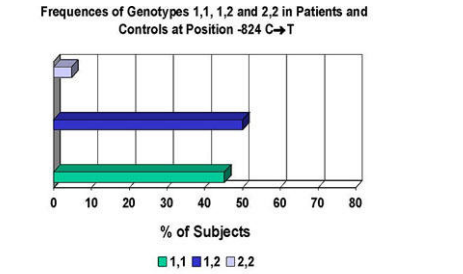

Fig. 3. The frequencies of genotypes 1,1, 1,2 and 2,2 at position –824 C→T in patients for the 3 disease groups, EOP, advanced AP (AAP), moderate AP (MAP) and controls (*n* = 62). Genotypes 1,1 *n* = 28; 1,2 *n* = 31 and 2,2 *n* = 3. The frequency of genotype 2,2 at position –824 C→T was significantly decreased in all groups compared to genotypes 1,1 and 1,2.

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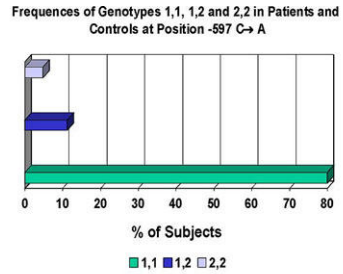

Fig. 4. The frequencies of genotypes 1,1, 1,2 and 2,2 at position –597 C→A in patients for the 3 disease groups, EOP, advanced AP (AAP), moderate AP (MAP) and controls (*n* = 62). Genotypes 1,1 *n* = 52; 1,2 *n* = 7 and 2,2 *n* = 3. The frequency of genotype 1,1 at position –597 C→A was significantly increased in all groups compared to genotypes 1,2 and 2,2.

Fig. 4. The frequencies of genotypes 1,1, 1,2 and 2,2 at position -597 C->A in patients for the 3 disease groups, EOP, advanced AP (AAP), moderate AP(MAP) and controls (n = 62). Genotypes 1,1 n = 52; 1,2 n = 7 and 2,2 n = 3.The frequency of genotype 1,1 a

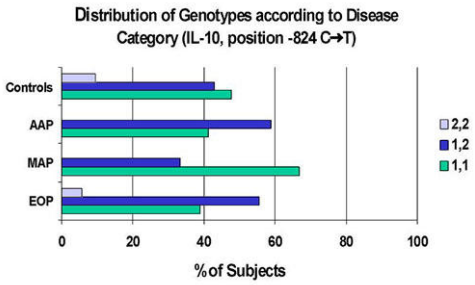

Fig. 5. The frequency of genotypes at position –824 containing allele *C and * T in the different disease groups. EOP *n* = 18, moderate AP (MAP) *n* = 6, advanced AP (AAP) *n* = 17, controls *n* = 21.

Fig. 5. The frequency of genotypes at position -824 containing allele *C and *T in the different disease groups. EOP n = 18, moderate AP (MAP) n = 6, advanced AP (AAP) n = 17, controls n = 21.

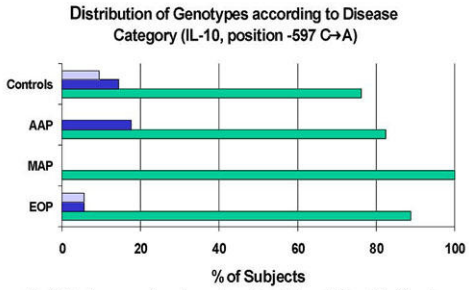

Fig. 6. The frequency of genotypes at position –597 containing allele *C and *A in the different disease groups. EOP *n* = 18, moderate AP (MAP) *n* = 6, advanced AP (AAP) *n* = 17, controls *n* = 21.

Fig. 6. The frequency of genotypes at position -597 containing allele *C and *A in the different disease groups. EOP n = 18, moderate AP (MAP) n = 6, advanced AP (AAP) n = 17, controls n = 21.

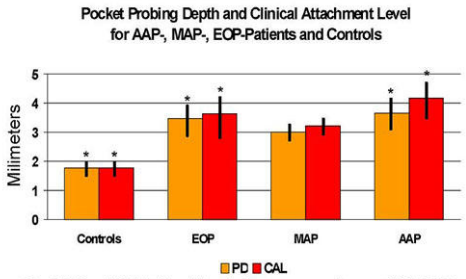

Fig. 7. PD and CAL in the different disease groups (mean ± SD). EOP *n* = 18, advanced AP (AAP) *n* = 17, controls *n* = 21. PD and CAL were significantly increased in EOP and AAP disease compared to controls.

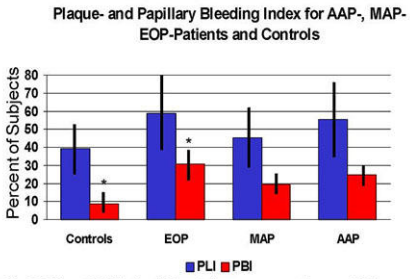

Fig. 8. PLI and PBI in the different disease groups (mean ± SD), EOP *n* = 18, advanced AP (AAP) *n* = 17, moderate AP (MAP) *n* = 6 and controls *n* = 21. PBI was significantly increased in EOP patients compared to controls.

Fig. 7. PD and CAL in the different disease groups (mean ± SD). EOP n = 18, advanced AP (AAP) n = 17, controls n = 21. PD and CAL were significantly increased in EOP and AAP disease compared to controls.

Fig. 8. PLI and PBI in the different disease groups (mean ± SD), EOP n = 18, advanced AP (AAP) n = 17, moderate AP(MAP) n = 6 and controls n = 21. PBI was significantly increased in EOP patients compared to controls.

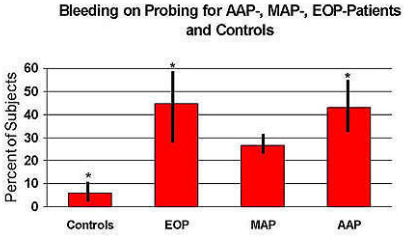

Fig. 9. BOP in the different disease groups (mean ± SD), EOP *n* = 18, advanced AP (AAP) *n* = 17, moderate AP (MAP) *n* = 6 and controls *n* = 21. BOP was significantly increased in EOP and AAP patients compared to controls.

Fig. 9. BOP in the different disease groups (mean ± SD), EOP n = 18, advanced AP (AAP) n = 17, moderate AP (MAP) n = 6 and controls n = 21. BOP wassignificantly increased in EOP and AAP patientscompared to controls.

Discussion and Conclusions

An association between IL-10 promoter polymorphisms and SLE has been reported. Allele *A at the -597 position and allele *T at the -824 position of the IL-10 gene were significantly associated with glomerulonephritis in southern Chinese patients (Mok et al. 1998). In the present study we investigated these two known IL-10 gene promoter polymorphisms in patients with AP, EOP and controls and their possible clinical associations. The frequencies found in our patients and controls were very similar with that previously reported by Turner et al. (1997) in white patients in the UK with systemic lupus erythematosus (LSM). We also observed an association between allele *A at position -597 and allele *T at position -824, but this association did not reach statistical significance, since only one patient (6.25%) and two controls carried this genotype. However, considering the wide spectrum of immunological functions in which this cytokine is involved, and their genetic association with systemic inflammatory diseases, we think that further genotyping of the IL-10 gene is necessary, in order to obtain an association with periodontitis.

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