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Interleukin-10 Gene Polymorphisms in Recurrent Aphthous Stomatitis

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Recurrent aphthous stomatitis (RAS) is a common oral inflammatory disease with unknown etiology in which the immune system seems to have a role in oral tolerance. Interleukin (IL)-10 is a cytokine synthesis inhibitory factor. Single nucleotide polymorphisms (SNPs) of *IL10* gene could alter this cytokine production. The aim of this study was to investigate frequencies of *IL10* alleles and genotypes in a group of individuals with RAS. Genomic DNA of 60 Iranian patients with RAS were typed for *IL10* gene (C/A −1082, C/T −819, and C/A −592), using PCR-SSP method. Frequency of each allele and genotype was compared to control group.
A significantly higher frequencies of the T allele at position −819 ($p = 0.006$) and the A allele at position of −592 ($p < 0.001$) were found in the patients with RAS group, when compared to the controls. *IL10* GA genotype at position −1082 ($p = 0.007$), CA genotype at position −592 ($p = 0.001$), and CT genotype at position −819 ($p = 0.001$) were significantly higher in the RAS patients. The results of this study suggest that certain SNPs of *IL10* gene have association with predisposition of individuals to RAS. However, further multicenter studies should be conducted to confirm the results of this study.

Keywords Interleukin 10, recurrent aphthous stomatitis, single nucleotide polymorphisms

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INTRODUCTION

Recurrent aphthous stomatitis (RAS) is a common human oral inflammatory disease, characterized by several episodes of small ulcers, which are usually painful (Preeti et al., 2011). Several environmental factors such as trauma, psychological stress, systematic disease, nutritional deficiencies, and allergies have been reported in association with RAS, while genetic factors could also prone individuals to this condition. However, despite several studies in this field, the exact etiology and pathogenesis of the disease has not been fully understood yet (Jurge et al., 2006).

Immunological pathways, especially cell-mediated immune response, was one of the main interests of scientists as underlying pathophysiology for RAS (Chavan et al., 2012). The immune responses are usually well controlled to prevent any excessive inflammatory reaction, while induction of oral tolerance could be associated with activation of certain T-helper (Th) cells (Smith et al., 2000). Interleukin (IL)-10, also known as cytokine synthesis inhibitory factor, is synthesized by Th2 cells and has pleiotropic effects in immune regulation and inflammation by down-regulating the expression of Th1 cytokines (Moore et al., 1993). Therefore, it could be a good candidate in oral tolerance (Garside et al., 1999; Smith et al., 2000), whereas SNPs of *IL10* could affect production of this cytokine. As production of IL-10 can be controlled by SNPs of *IL10* gene, frequencies of *IL10* alleles and genotypes were studied in a group of subjects with RAS.

MATERIALS AND METHODS

Subjects

Sixty-four Iranian patients with RAS (24 male and 40 female), with mean age of 36.6 years (range of 20 to 61 years), were included in this study. Among them, 23 cases had less than 3 episodes of aphthous per month, while the remaining 41 cases had 3 or more than 3 episodes of aphthous per month. Indeed the results were compared to 140 ethnicity-matched control subjects from Tehran. Both the study and control groups were from the same geographic area in Tehran, Iran. The diagnosis of RAS was made based on history, clinical manifestations, and histopathology features, including at least three attacks within past three years. The diagnosis of RAS was based on accepted clinical criteria (Ship et al., 2000). The exclusion criteria for both study and control groups were any other diseases such as Behcet's disease, exposure to radiation and drug consumption, systemic lupus erythematosus, celiac disease, Sweat's syndrome, inflammatory bowel disease, Reiter syndrome, PFAPA syndrome, HIV infection, tumors of the oral cavity, presence of pregnancy and periodontal disease.

Genotyping

Five mL blood was collected in EDTA tubes. DNA was isolated, using a phenol-chloroform method. IL-10 gene typing was performed, using polymerase chain reaction with sequence-specific primers (PCR-SSP, Heidelberg University, Germany); the technique was previously explained in details (Amirzargar et al., 2008). The allele frequencies of *IL10* (C/A −1082 [rs1800896], C/T −819

[rs1800871], and C/A −592 [rs1800872]) were studied. The internal positive control primer pairs included in the PCR-SSP primer mix for typing of the positions amplify a 90 base pair (bp) fragment of the human β-globin gene. In addition, we used double-distilled water (DDW) for the negative control of each test. Data including alleles, genotypes, and haplotypes were analyzed, based on the manufacturer instructions (Product No: 124, Lot No: CYT20).

Statistics

Allele and genotypes frequencies were counted directly. The results found for alleles, genotypes and haplotypes frequencies in patients were compared to control groups, using chi-square test. The odds ratio (OR) and 95% confidence intervals (9%CI) were calculated. P values (p) of less than 0.05 were considered significant. The Bonferroni multiple comparison correction method was utilized in computing of confidence intervals and rejection of tests at the 0.05 level. Thus, the resultant p value of less than 0.008 was considered as significant for genotypes and for haplotypes.

RESULTS

The results of *IL10* allelic and genotype frequencies in RAS patients and the control group are presented in Table 1. T allele at position −819 ($p = 0.006$) and A allele at position of −592 ($p < 0.001$) were significantly overrepresented in the patient group, compared to the controls.

Although frequency of GA genotype at position −1082 in RAS was significantly lower than the controls ($p = 0.007$), no significant difference

Table 1. Comparison of alleles, genotypes and haplotypes frequencies of *IL10* between patients with RAS and the control group.

Position	Alleles/ Genotypes/ Haplotypes	RAS ($n = 60$), n (%)	Controls ($n = 140$), n (%)	p Value	Odds Ratio (95% Confidence Interval)
−1082	A	73(60.8)	181(64.6)	0.54	0.85(0.53–1.35)
	G	47(39.2)	99(35.4)	0.54	1.18(0.74–1.87)
	AA	14(23.3)	53(37.8)	0.0671	0.50(0.24–1.04)
	GA	45(75)	75(53.6)	0.0074*	2.60(1.27–5.39)
	GG	1(1.7)	12(8.6)	0.0576	0.18(0.01–1.39)
−819	C	64(56.1)	199(71.1)	0.0062	0.52(0.32–0.84)
	T	50(43.9)	81(28.9)	0.0062	1.92(1.19–3.09)
	CC	13(22.8)	71(50.7)	0.0005*	0.29(0.13–0.61)
	CT	38(66.7)	57(40.7)	0.0016*	2.91(1.46–5.85)
	TT	6(10.5)	12(8.6)	0.873	1.25(0.39–3.86)
−592	A	55(47.4)	81(28.9)	0.0006	2.22(1.38–3.55)
	C	61(52.6)	199(71.1)	0.0006	0.45(0.28–0.72)
	AA	8(13.8)	12(8.6)	0.395	1.71(0.59–4.83)
	CA	39(67.2)	57(40.7)	0.0012*	2.99(1.50–6.00)
	CC	11(19)	71(50.7)	<0.0001*	0.23(0.10–0.50)
−1082, −819, −592	GCC	45(45.9)	99(35.4)	0.083	1.55 (0.95–2.54)
	ACC	10(10.2)	100(35.7)	<0.0001*	0.2 (0.1–0.43)
	ATA	43(43.9)	81(28.9)	0.0096	1.92 (1.16–3.18)

*Significant at the 5% level adjusted for Bonferroni multiple comparison correction.

on alleles and other genotypes frequencies between two groups was found (Table 1). *IL10* CA genotype at position −592 ($p = 0.001$) and CT genotype at position −819 ($p = 0.001$) in the RAS patients were both significantly higher than controls, while *IL10* CC genotype at positions −592 and −819 were significantly decreased in RAS ($p < 0.001$) (Table 1).

The haplotype frequencies of IL-10 in the patients with RAS and the controls are also presented in Table 1, which shows significantly lower frequency of ACC haplotype in the patient group.

DISCUSSION

Association of *IL10* SNPs with several diseases has been documented (Amirzargar et al., 2005, 2006; Barkhordari et al., 2010; Bashashati et al., 2012; Movahedi et al., 2008; Rezaei et al., 2010). However, its role in RAS has been uncertain so far. In a study by Buno et al. (1998), there was not any significant increase in IL-10 mRNA of RAS lesions; meantime IL-2, IFN-gamma and TNF-alpha mRNA levels were significantly increased, which was consistent with a cell-mediated immune response. Moreover, lower IL-10 mRNA levels were reported in the clinically normal mucosa of patients with RAS. Therefore IL-10 defect in the oral mucosa, which could lead to failure in suppressing the inflammatory reaction, is suggested as a pathogenesis in RAS (Buno et al., 1998).

In this study, we investigated the genetic polymorphisms of *IL10* gene; significant differences in frequencies of alleles and genotypes frequencies were found between the group of patients with RAS and the control group. The polymorphisms of *IL10* at positions −1082 (C/A), −819 (C/T), and −592 (C/A) were investigated in RAS patients and healthy controls, while these polymorphisms are all located in the promoter region of *IL10*, on chromosome 1q31-32. Carriage of the *IL10* −1082 G allele seems to be associated with higher production of IL-10 *in vitro* and the −1082 A allele is associated with lower production of IL-10 (Turner et al., 1997). In our investigation, the −1082 GA genotype was significantly higher in the patient group, which is in contrast with previous studies (Bazrafshani et al., 2003; Guimaraes et al., 2007). Such conflicting results could be related to the differences in the study design and the population heterogeneity. More studies in larger groups and among different ethnicities are needed for identifying the responsible polymorphisms for the functional deficiency of IL-10 in patients with RAS. Indeed the −819 CT and −592 CA genotypes were significantly higher in RAS in our study, while the −819 CC and −592 CC genotypes were significantly lower than control group. Further multi-center studies with large number of patients could be recommended to confirm the results of this study. Measurement of IL-10 production and its association of *IL10* SNPs could also be suggested in subsequent projects.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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