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ORIGINAL ARTICLE

Tumour necrosis factor-alpha gene polymorphisms and susceptibility to oral lichen planus

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OBJECTIVE: This study is aimed to investigate the association between OLP susceptibility and clinical type in the Thai population and three polymorphisms within the promoter region of the *TNF*-α at positions -863, -308 and -238 which have putative functional significances.

MATERIALS AND METHODS: Genomic DNA from 75 Thai patients with OLP and 154 healthy controls were genotyped for *TNF*-α polymorphisms – -863(rs1800630), -308(rs1800629), and -238(rs361525) – using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

RESULTS: We found a higher proportion of *TNF-alpha*-308 AA genotype (high producer genotype) among OLP patients (5/75; 6.67%) when compared to healthy controls (1/154; 0.65%; OR = 10.93; 95% CI = 1.21–251.9). For other polymorphisms (-863 and -238), we did not find any significant association with OLP development; this was also the case with haplotype analysis (-863/-308/-238).

CONCLUSION: *TNF*-α-308AA may play a relevant role in the susceptibility and severity of OLP in the Thai population. However, further investigation of this study is needed.

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Keywords: oral lichen planus; polymorphisms; tumour necrosis factor-alpha

Introduction

Oral lichen planus (OLP) is an immune-mediated disease that affects the oral mucous membranes. This effect leads to a variety of clinical presentations, with the main clinically distinct types recognized as erosive and non-erosive. The prevalence in the general population is approximately 0.1–4% (McCartan and Healy, 2008). The usual onset occurs in middle-aged persons, and is more common in females (Lodi *et al*, 2005). The aetiogenesis of OLP remains elusive. However, several studies suggest that immunological mechanisms are involved in the pathogenesis of OLP (Regezi *et al*, 1978; Sugerman *et al*, 1992; Porter *et al*, 1997). Immunohistological studies have found increased T-lymphocytic infiltration in OLP lesions. The majority of OLP-related T-cells within the epithelium and adjacent to damaged basal keratinocytes are activated cytotoxic $CD8^+$ T-cells (Kilpi, 1987; Jungell *et al*, 1989; Sugerman *et al*, 2000). Furthermore, $CD8^+$ T-cells co-localize with apoptotic keratinocytes in OLP lesions (Sugerman *et al*, 2000; Khan *et al*, 2003). A possible mechanism for $CD8^+$ cytotoxic T-cells to trigger keratinocyte apoptosis is TNF-α release for binding to TNF-α receptor 1 (TNFR1) on the keratinocyte surface (Lodi *et al*, 2005).

TNF-α is a cytokine secreted from various cells such as activated monocytes, macrophages, B cells, T cells, mast cells and fibroblasts (Vilcek and Lee, 1991; Vassalli, 1992).Its functions are involved in inflammation, immune response and apoptosis (Banno *et al*, 2004). TNF-α has been reported to be up-regulated in lesional T cells and serum from OLP patients as compared with controls (Simark-Mattsson *et al*, 1999; Sklavounou-Andrikopoulou *et al*, 2004). These data suggest that TNF-α might be involved in the pathogenesis of OLP. Previous studies showed that genetic polymorphism of *TNF*-α at the promoter region -308 are associated with OLP (Carrozzo *et al*, 2004; Xavier *et al*, 2007). In addition, a disease severity study of Bai *et al* (2009) reported that the frequencies of *TNF*-α -308A allele in patients with erosive OLP were significantly greater than in the control group. Independent association studies are still needed to confirm or disprove previous finding. Furthermore, another important SNP at –863 (which has been suggested to influence

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TNF-α expression, possibly via allele-differential binding to nuclear factor (NF)-κB with an effect on chromatin remodeling (Skoog *et al*, 2006) has not been reported in OLP before. Thus, the aim of this study was to analyse the association between the three commonly studied single nucleotide polymorphisms (SNP) -863(rs1800630), -308(rs1800629) and -238 (rs361525) within the *TNF*-α which have putative functional significances, and the susceptibility to and/or severity of OLP in the Thai population.

Materials and methods

Subjects

Two hundred and forty-five paraffin-embedded samples were diagnosed for OLP between January 1999 and December 2008 by the Department of Oral Pathology, Faculty of Dentistry, Mahidol University. The OLP diagnosis was confirmed by an oral pathologist using the WHO diagnostic criteria of lichen planus (Kramer *et al*, 1978). According to the limitations of clinical examination, we considered OLP cases based on the histological features of OLP. All historical data and clinical manifestations were reviewed from each patient's chart; this information was transferred onto a clinical data sheet. Clinical and histology appearances of secondary candida infection were not investigated. Totally, 170 samples were excluded (145 samples with deficiency in the cell amount for DNA extraction and 25 samples with historical and clinical suspicion of lichenoid reaction). Lichenoid reaction was suspected based on the history of medication, direct contact with dental restorative materials, and history of grafting or graft-versus-host disease (Al-Hashimi *et al*, 2007). DNA extraction of good quality was successfully achieved in only 75 cases. These underwent genotyping for all three SNPs; the genotype rate was 100%. This OLP group consisted of 62 women and 13 men (mean age ± SD = 49.87 ± 14.99 years); 24 were classified as erosive subtype and 51 as non-erosive subtype, based on their chart records. Erosive OLP has clinical diagnoses together with the biopsy of erythema or ulcerations (Edwards and Kelsch, 2002).

For a control group, we obtained blood samples from 154 healthy Thai people (57 women and 97 men; mean age ± SD = 30.9 ± 10.6 years) recruited from locations near Mahidol University from June to August 2009. All of them were asked to fill out a questionnaire in order to exclude individuals with oral lesions. All the subjects participating in the study belonged to the Thai ethnicity, from the central area of Thailand. The ethics committee of Mahidol University, Bangkok, Thailand, approved the study, and the healthy control subjects gave their informed consent.

DNA extraction

Extraction of genomic DNA from formalin-fixed paraffin-embedded tissue was performed using a MagneSil® Genomic, Fixed Tissue System kit (Promega, Madison, WI, USA). For blood samples, DNA was extracted from the buffy coat collected with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, using a salting-out method (Miller *et al*, 1988). DNA was aliquoted and stored at −20°C until used. The DNA from both the OLP and healthy control groups were collected anonymously.

Genotyping study

A genotyping study was performed blindly at the Lupus Research Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to analyse the *TNF*-α at promoter positions -863 (A/C), -308 (A/G) and -238 (A/G), as previously described (Allen *et al*, 2001; Wennberg *et al*, 2002; Lu *et al*, 2004). Negative controls without DNA template were included in each experiment. Ten percent of the samples were confirmed by direct sequencing of PCR products to verify the accuracy of genotyping.

Statistical analysis

Genotype frequencies were checked for consistency among normal controls with those expected from the Hardy–Weinberg equilibrium (HWE). Allele and genotype frequencies were compared between groups using the chi-squared (χ^2) test or Fisher's exact probability test, where appropriate. The PLINK v1.07 program (Purcell *et al*, 2007) was used to calculate HWE, *P*-values, odds ratios and 95% confidence intervals, as well as for haplotype analysis. *P*-values were adjusted by Bonferroni correction for the number of SNPs. A corrected *P*-value (*Pc*) of <0.05 was considered statistically significant. The power for our genetic association study was calculated using the PS program (Dupont and Plummer, 1990), based on our sample size (75 cases *vs* 154 controls), probability of exposure among controls (0.019) (Xavier *et al*, 2007) and an odds ratio of 9.41 (Xavier *et al*, 2007). By this calculation, our study had a power of 91.1%, with $\alpha = 0.05$.

Results

The distribution of genotype and allele frequencies of *TNF*-α polymorphisms in OLP patients and control subjects are shown in Table 1. In the present study, all SNPs were in Hardy–Weinberg equilibrium when comparing the observed and expected genotype frequencies of each SNP ($P > 0.05$). There was no significant difference in *TNF*-α-308 A alleles among the OLP patients and controls. However a significant increase of the *TNF*-α-308 AA genotype was observed in the group of OLP patients when compared to healthy controls: 5 (6.67%) *vs* 1 (0.65%); OR = 10.93 and $P = 0.015$, $Pc = 0.045$. Moreover, we found a slight difference of AA genotype between erosive OLP patients and healthy controls: 2 (8.33%) *vs* 1 (0.65%); OR = 13.91 and $P = 0.048$. However, this was not statistically significant when corrected for multiple comparisons ($Pc = 0.144$). Besides, please note that multiple comparisons were performed. For the *TNF*-α-308 AG

Table 1 Genotype and allele frequencies of TNF-α gene polymorphisms in OLP patients and control subjects

SNP	*Genotype/ allele*	*Total OLP* n *(%)*, n = *75*	*Erosive OLP n* (%), n = *24*	*Non-erosive OLP* n *(%)*, n= *51*	*Healthy control* n *(%)*, n = *154*
-863 (rs1800630)	AA	5 (6.67)	1 (4.17)	4 (7.84)	6 (3.90)
	AC	8 (10.67)	3 (12.5)	5 (9.80)	35 (22.72)
	CC	62 (82.66)	20 (83.33)	42 (82.35)	113 (73.38)
	A	18 (12)	5 (10.42)	13 (12.75)	47 (15.26)
	C	132 (88)	43 (89.58)	89 (87.25)	261 (84.74)
-308 (rs1800629)	AA	5 (6.67)[a]	2 (8.33)[b]	3 (5.88)	1 (0.65)
	AG	7 (9.33)	1 (4.17)	6 (11.76)	28 (18.18)
	GG	63 (84)	21 (87.5)	42 (82.35)	125 (81.17)
	A	17 (11.33)	5 (10.42)	12 (11.76)	30 (9.74)
	G	133 (88.67)	43 (89.58)	90 (88.24)	278 (90.26)
-238 (rs361525)	AA	(0)	0 (0)	0 (0)	0 (0)
	AG	8 (10.67)	2 (8.33)	6 (11.76)	11 (7.14)
	GG	67 (89.33)	22 (91.67)	45 (88.24)	143 (92.86)
	A	8 (5.33)	2 (4.17)	6 (5.88)	11 (3.57)
	G	142 (94.67)	46 (95.83)	96 (94.12)	297 (96.43)

OLP, oral lichen planus; SNP, single nucleotide polymorphism.
[a] AA compared with AG+ GG genotype (total OLP *vs* healthy control); OR (95% CI) = 10.93 (1.21–251.9), $P = 0.015$ ($Pc = 0.045$).
[b] AA compared with AG+GG genotype (erosive OLP *vs* healthy control); OR (95% CI) = 13.91 (0.93–405.83), $P = 0.048$ ($Pc = 0.144$).

genotype, no significant associations were found in the groups of total OLP and erosive OLP when compared to healthy controls: 7 (9.33%) and 1 (4.17%) *vs* 28 (18.18%). We also did not find any significant association of other polymorphisms (-863 and -238) with OLP development.

In addition, we performed haplotype analysis of SNPs -863, -308 and -238 in *TNF*-α. There were four common haplotypes (minor haplotype frequency ≥0.05) including CGG, AGG, CAG and CGA. Our results showed no significant association between haplotypes and OLP development (data not shown).

Discussion

In the present study, the AA genotype of *TNF*-α at position -308 represented a tenfold increased risk (OR = 10.93) in OLP development when compared to a combination of the AG and GG genotypes. We did not find a *TNF*-α-308 AG genotype difference between OLP and controls ($P = 0.081$, $Pc = 0.243$), as suggested previously by Carrozzo *et al* (2004). However, although with limited sample size, we found five OLP patients with a *TNF*-α-308 AA genotype as compared to one of the controls. This is consistent with a previous study which found an association of AA genotype with OLP (Xavier *et al*, 2007). This SNP involves the alteration of an amino acid from adenine (allele A) into guanine (allele G). The A allele of SNP-308 has been shown to be associated with higher *TNF*-α production (Brinkman *et al*, 1995; Jongeneel and Beutler, 1995; Abraham and Kroeger, 1999). Furthermore, we observed an association of the *TNF*-α-308 AA genotype with an increased risk of erosive OLP development (OR = 13.91), although this was not statistically significant when corrected for multiple comparisons. Two previous studies did not find any association between *TNF*-α polymorphism and severity of OLP (Carrozzo *et al*, 2004; Xavier *et al*, 2007). However a recent study by Bai *et al* (2009) showed that the *TNF*-α-308A allele was associated with erosive OLP when compared to controls. Erosive OLP is more often associated with symptoms than non-erosive OLP (Edwards and Kelsch, 2002). One study showed that apoptosis in erosive OLP was more intense than in non-erosive OLP, and that both forms presented more apoptosis than healthy oral mucosa (Brant *et al*, 2008). It is possible that the *TNF*-α-308 AA genotype influences the higher production of *TNF*-α, leading to more keratinocyte apoptosis in erosive OLP patients.

In addition, haplotype analysis (-863/-308/-238) showed no significant association with OLP. These data support the importance of *TNF*-α-308 polymorphism in OLP. Because *TNF*-α is part of the MHC class III cluster on the short arm of chromosome 6, the effect of *TNF*-α might be due to the linkage disequilibrium with some HLA alleles. Carrozzo *et al* (2004) reported that the -308A allele was in linkage disequilibrium with HLA-DR3 and HLA-DR6. In that study, they found no significant association between HLA-DR and OLP patients. However their study was performed on a Caucasian population. A further association study of HLA-DR and OLP patients in a Thai population is required.

Our study had some limitations. Firstly, oral examinations were not performed on the control groups. Secondly, our study had limited sample size and unmatched age and gender of the participants between the OLP and the control groups due to a lack of genotyping data from a number of samples. This problem was a limitation mainly from the use of paraffin-embedded formalin-fixed tissue samples that did not allow complete DNA extraction and amplification.

In conclusion, we found an association between *TNF*-α-308 polymorphism and OLP patients, particularly in patients with the erosive form. Based on these data, we suggest that *TNF*-α-308 polymorphism may be involved in the susceptibility and severity of OLP in the Thai population.

Author contributions

I. Kimkong contributed to the study design, definition of intellectual content, experimental studies, data analysis and manuscript preparation. N. Hirankarn contributed to manuscript editing and reviews; J. Nakkuntod conducted the experiments and data acquisition; and N. Kitkumthorn contributed to the study concepts, study design and manuscript editing.

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