IL-10 and *TNF-α* promoter polymorphisms in susceptibility to systemic lupus erythematosus in Taiwan

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Abstract

Objective

The genetic control of Interleukin-10 (IL-10) and Tumour necrosis factor-α (TNF-α) production and the possible interaction between the two cytokines in influencing SLE susceptibility as well as clinical features has not been completely evaluated in the Taiwanese population.

Methods

We investigated the association of IL-10 and TNF-α promoter polymorphisms (-1082, -819 and -592 for IL-10 gene; -308 for TNF-α gene) with SLE in a total of 172 Taiwanese patients and 215 controls.

Results

Our results indicate that IL-10 A/T/A-A/T/A genotype was associated with Taiwanese SLE, whereas no significance was observed between TNF-α genotype and SLE. Furthermore, the TNF-α G allele frequency of the polymorphism at -308 was significantly decreased in patients with oral ulcers. The combined frequencies of IL-10 A/T/A haplotype and TNF-α G-G genotype were significantly increased in SLE patients. In addition, the combined frequencies of IL-10 A/T/A haplotype and TNF-α G-G genotype were significantly decreased in patients with oral ulcers.

Conclusion

These results suggest a significant correlation of the combined IL-10 and TNF-α genetic polymorphisms contribute to SLE susceptibility and clinical features in the Taiwanese population.

Key words

Systemic lupus erythematosus, interleukin-10, tumour necrosis factor-α, single nucleotide polymorphism

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Introduction

Systemic lupus erythematosus (SLE), is a systemic autoimmune disorder, characterised by distinct clinical manifestations (1-4). Although the exact etiology and pathogenesis of SLE are unknown, it is believed that the disease is genetic in origin (2). Cytokines are potent immunomodulatory molecules that mediate immune response and inflammation. Investigators have explored the role of several cytokines involved in pathogenesis of SLE (5-7).

Interleukin-10 (IL-10), produced by monocytes and lymphocytes, is a potent cytokine that has pleiotropic effects in immunoregulation and inflammation (8, 9). It has been reported that the serum levels of IL-10 are elevated in the case of SLE patients (5, 10). IL-10 production appears to be influenced by the polymorphisms in the *IL-10* promoter region (11, 12). In addition, the three *IL-10* promoter SNPs are strongly associated with the pathogenesis of SLE (13-18). Tumour necrosis factor-α (*TNF*-α), an important proinflammatory cytokine, exerts a variety of physiologic and pathogenic effects, including the activation of a cascade of inflammatory events, which lead to tissue destruction in autoimmune diseases (19-21). Levels of *TNF*-α have been reported in SLE patients and have been shown to correlate with SLE disease activity (22-24). Nevertheless, studies on *TNF*-α genetic polymorphisms in SLE patients have yielded inconclusive results (25-31). The production of these two regulators of the inflammatory reactions-IL-10 and *TNF*-α has been found to be deeply deregulated in SLE, suggesting that these regulators may be involved in the pathogenesis of SLE.

The genetic control of *IL-10* and *TNF*-α production and the possible interaction between the two cytokines in influencing SLE susceptibility as well as clinical features has not been completely evaluated in the Taiwanese population. Our aim in the present study was to investigate the influence of cytokine genetic variants and their interactions in Taiwanese SLE population.

Patients and methods

Study population

The study subjects including a total of 172 patients with SLE and 215 healthy subjects were recruited from China Medical University Hospital in Taiwan. However, the total numbers for SLE patients or controls illustrated in Table I do not agree with each other for the *IL-10* and *TNF*-α may due to the poor quality of the genomic DNA of SLE patients or controls. The poor quality of the genomic DNA may lead to PCR failure. Therefore, the total numbers for SLE patients or controls in Table I mean the success in PCR reaction numbers. Furthermore, due to insufficient information of SLE clinical features, some patients may only have genotyping results without clinical features; the numbers of SLE patients with various clinical features regarding genetic haplotypes may be inconsistent. All of the patients met American Rheumatism Association criteria for SLE classification (32). The healthy individuals from the general population were also enrolled. Informed consent was obtained from each patient and control subjects involved. DNA collection was approved by China Medical University Hospital's Ethics Board.

IL-10 *and* TNF-α *promoter polymorphisms genotyping*

Genomic DNA was extracted from peripheral blood leukocytes according to standard protocols (Roche Genomic DNA kit). The 3 biallelic *IL-10* promoter polymorphisms were detected by TaqMan(R) Genotyping Assays (Applied Biosystems) (Supplemental Table I). The *TNF*-α genetic polymorphism (-308) were detected by PCR using primers that amplified a short fragment of DNA containing the polymorphism (Supplemental Table II). Polymorphic site identification was performed by incubating the PCR products with a restriction enzyme chosen to cut 1 of the 2 alleles, followed by electrophoresis on 3% agarose gels. All samples were amplified and digested in parallel with 3 samples of a known genotype.

Statistical analysis

Polymorphism genotype frequencies and allelic frequency distributions in SLE patients and control individuals were analysed with chi-square tests (SPSS Version 10.0). Statistical signifi-

Table I. Distribution of IL-10 and TNF-α promoter genotype in Taiwanese SLE patients and controls.

Promoter genotypes IL-10 (-1082/-819/-592)	SLE Number (%)	Controls Number (%)	*p*-value	Odds ratio (95% CI)
A/C/C-A/C/C	1 (0.6)	7 (3.3)	0.066	0.17 (0.02–1.43)
A/C/C-G/C/C	0 (0.0)	3 (1.4)	0.120	ND
A/T/A-A/C/C	56 (32.6)	84 (39.1)	0.185	0.75 (0.49–1.15)
A/T/A-A/T/A	96 (55.8)	78 (36.3)	**<0.001***	2.22 (1.47–3.34)
A/T/A-A/T/C	2 (1.2)	8 (3.7)	0.115	0.30 (0.06–1.45)
A/T/A-G/C/C	13 (7.6)	13 (6.0)	0.555	1.27 (0.57–2.82)
A/T/C-A/C/C	2 (1.2)	16 (7.4)	**0.004***	0.15 (0.03–0.65)
A/T/C-A/T/C	1 (0.6)	1 (0.5)	0.874	1.25 (0.08–20.15)
A/T/C-G/C/C	1 (0.6)	5 (2.3)	0.168	0.25 (0.03–2.12)
A/T/A	167 (97.1)	174 (80.9)	**<0.001***	7.87 (3.04–12.00)
Non A/T/A	5 (2.9)	41 (19.1)		1.00
TNF-α (-308)				
A-A	3 (1.9)	2 (0.9)	0.747	1.98 (0.33–12.00)
G-A	26 (16.1)	35 (16.6)		0.98 (0.56-1.71)
G-G	132 (82.0)	174 (82.5)		1.00
-308 A allele	32 (9.9)	39 (9.2)	0.749	1.08 (0.66–1.77)
-308 G allele	290 (90.1)	383 (90.8)		1.00
G-G	132 (82.0)	174 (82.5)	0.905	1.03 (0.60–1.77)
Non G-G	29 (18.0)	37 (17.5)		1.00

The significance was evaluated by χ^2 test or two-tailed Fisher's exact test.
IL-10: interleukin-10; TNF-α: tumour necrosis factor-α; SLE: systemic lupus erythematosus; 95% CI: 95% confidence intervals; ND: not determined.

cance was assumed at $p<0.05$. Allelic frequencies were expressed as percentages of total allele numbers. Odds ratios (OR) were calculated from genotype frequencies and allelic frequencies at a 95% confidence interval (CI). Haplotypes were inferred from unphased genotype data with Bayesian statistics (Phase 2.1 software) (33, 34). Adherence to the Hardy-Weinberg equilibrium constant was examined using a χ^2 test with one degree of freedom. The corrected *P* (*P*c) values were adjusted by using Bonferroni's correction for multiple comparisons. Statistical significance was considered as *P*c-value <0.0045 (0.05/11 clinical features).

Results

IL-10 and TNF-α promoter polymorphisms

The promoter genotype frequencies of *IL-10* and *TNF-α* promoter polymorphisms are shown in Table I. Nine *IL-10* promoter haplotypes were present in both SLE patients and controls. No statistically significant differences were observed in *IL-10* promoter haplotypes between these two groups with two exceptions: A/T/A-A/T/A and A/T/C-A/C/C. In SLE patients, A/T/A-A/T/A genotype frequency was significantly higher ($p<0.001$; OR=2.22, 95% CI=1.47-3.34) and A/T/C-A/C/C frequency was significantly lower ($p=0.004$; OR=0.15, 95% CI=0.03-0.65). Observed frequencies for individuals carrying the A/T/A haplotype were 97.1% in SLE patients and 79.1% in controls ($p<0.001$; OR=8.83, 95% CI=3.42-22.76). No significant difference in the allele and genotype frequencies of the -308 position of *TNF-α* promoter gene was observed between SLE patients and controls.

IL-10, TNF-α promoter polymorphisms and clinical features of SLE

The association between the clinical feature profile of SLE patients with

Table II. IL-10 haplotype frequencies of Taiwanese SLE patients with various clinical features.

Clinical features	Allele (n=264)			Genotype (n=132)			
	Ht1, n=196 Number (%)	Non-Ht1, n=68 Number (%)	Pc value	Ht1/Ht1, n=69 Number (%)	Ht1/Non-Ht1, n=58 Number (%)	Non-Ht1/Non-Ht1, n=5 Number (%)	*Pc*-value
ANA	192 (98.0)	66 (97.1)	0.668	68 (98.6)	56 (96.6)	5 (100.0)	0.709
Immunologic disorder	150 (76.5)	56 (82.4)	0.318	50 (72.5)	50 (86.2)	3 (60.0)	0.108
Haematological disorder	99 (50.5)	33 (48.5)	0.778	35 (50.7)	29 (50.0)	2 (40.0)	0.898
CNS disorder	23 (11.7)	11 (16.2)	0.346	8 (11.6)	7 (12.1)	2 (40.0)	0.181
Renal disorder	81 (41.3)	29 (42.6)	0.849	29 (42.0)	23 (39.7)	3 (60.0)	0.673
Serositis	40 (20.4)	14 (20.6)	0.975	14 (20.3)	12 (20.7)	1 (20.0)	0.998
Arthritis	105 (53.6)	41 (60.3)	0.337	35 (50.7)	35 (60.3)	3 (60.0)	0.542
Oral ulcers	50 (25.5)	26 (38.2)	0.046	20 (34.5)	20 (34.5)	3 (60.0)	0.083
Photoseneitivity	87 (44.4)	35 (51.5)	0.313	27 (46.6)	27 (46.6)	4 (80.0)	0.286
Discoid rash	27 (13.8)	13 (19.1)	0.290	9 (15.5)	9 (15.5)	2 (40.0)	0.266
Malar rash	99 (50.5)	39 (57.4)	0.330	31 (53.4)	31 (53.4)	4 (80.0)	0.402

Ht1: A/T/A; N: number of SLE patients in genotype analysis; NS: not signifificant; ANA: anti-nuclear antibodies.
The chi-square test (2x2 table for haplotype; 2x3 table for genotype) was performed to obtain the *p*-value. SLE patients with ht1 were compared with SLE patients without ht1 after stratification by clinical features. The corrected P (*Pc*) values were adjusted by using Bonferroni's correction for multiple comparisons. Statistical significance was considered as *Pc*-value <0.0045 (0.05/11 clinical features).

Table III. TNF-α-308 genetic polymorphism of Taiwanese SLE patients with various clinical features.

Clinical features	Allele frequency*			Genotype frequency*			
	A allele (n=32) Number (%)	G allele (n=290) Number (%)	*P*c value	A-A (n=3) Number (%)	A-G (n=26) Number (%)	G-G (n=132) Number (%)	*P*c-value
ANA	26 (81.3)	244 (84.1)	0.674	2 (66.7)	22 (84.6)	111 (84.1)	0.715
Immunologic disorder	23 (71.9)	197 (67.9)	0.649	2 (66.7)	19 (73.1)	89 (67.4)	0.850
Haematological disorder	9 (28.1)	131 (45.2)	0.065	0 (0.0)	9 (34.6)	61 (46.2)	0.170
CNS disorder	5 (15.6)	29 (10.0)	0.326	1 (33.3)	3 (11.5)	13 (9.8)	0.418
Renal disorder	15 (46.9)	103 (35.5)	0.206	1 (33.3)	13 (50.0)	45 (34.1)	0.304
Serositis	10 (31.3)	46 (15.9)	0.029	1 (33.3)	8 (30.8)	19 (14.4)	0.101
Arthritis	17 (53.1)	133 (45.9)	0.434	2 (66.7)	13 (50.0)	60 (45.5)	0.713
Oral ulcers	15 (46.9)	65 (22.4)	**0.002***	2 (66.7)	11 (42.3)	27 (20.5)	0.015
Photoseneitivity	18 (56.3)	108 (37.2)	0.037	1 (33.3)	16 (61.5)	46 (34.8)	0.038
Discoid rash	8 (25.0)	32 (11.0)	0.023	1 (33.3)	6 (3.8)	13 (9.8)	0.094
Malar rash	20 (62.5)	124 (42.8)	0.033	1 (33.3)	18 (69.2)	53 (40.2)	0.023

n: number of SLE patients in genotype analysis; NS: not significant; ANA: antinuclear antibodies.
χ^2 test (2x2 table for allele frequency; 2x3 table for genotype frequency) were performed to obtain the *p*-value. The corrected P (*P*c) values were adjusted by using Bonferroni's correction for multiple comparisons. Statistical significance was considered as *P*c-value <0.0045 (0.05/11 clinical features).
**P*c-value was <0.0045.

Table IV. IL-10 and TNF-α genotypes in Taiwanese SLE susceptibility.

		SLE patients		
	Controls, n. (%)	n. (%)	*p*-value	Odds ratio (95% CI)
Combined IL-10 and TNF-α	n=211	n=161		
A/T/A *vs*. G-G	143 (68.1)	128 (79.5)	**0.012***	1.84 (1.14-2.98)
A/T/A *vs*. non G-G	25 (11.9)	29 (18.0)	0.094	0.61 (0.34-1.09)
Non A/T/A *vs*. G-G	30 (14.3)	4 (2.5)	**<0.001***	0.15 (0.05-0.45)
Non A/T/A *vs*. non G-G	12 (5.7)	0 (0.0)	**0.002***	ND
Trend test			**<0.001***	

IL-10: interleukin-10; TNF-α: tumour necrosis factor-α; SLE: systemic lupus erythematosus; 95% CI: 95% confidence intervals; ND: not determined. **p* value was <0.05.

Table V. Influence of TNF-α-308 genotype on the clinical features of Taiwanese SLE patients with IL-10 ATA genotype.

Clinical features	TNF-α G-Gq (total n=107) Number (%)	TNF-α non G-G (total n=25) Number (%)	*P*c-value
ANA	104 (97.2)	24 (96)	0.753
Immunologic disorder	86 (80.37)	20 (80)	0.966
Haematological disorder	58 (54.21)	8 (32)	0.046
CNS disorder	11 (10.28)	4 (16)	0.417
Renal disorder	43 (40.19)	14 (56)	0.151
Serositis	19 (17.76)	9 (36)	0.045
Arthritis	56 (52.34	14 (46)	0.741
Oral ulcers	25 (23.36)	13 (52)	**0.004**[#]
Photoseneitivity	43 (40.19)	16 (64)	0.031
Discoid rash	12 (11.21)	7 (28)	0.031
Malar rash	50 (46.73)	18 (72)	0.023

χ^2 test (2x2 table for allele frequency; 2x3 table for genotype frequency) were performed to obtain the *p*-value. The corrected P (*P*c) values were adjusted by using Bonferroni's correction for multiple comparisons. Statistical significance was considered as *P*c-value <0.0045 (0.05/11 clinical features).
[#]*P*c-value was <0.0045

various alleles and genotypes of *IL-10* promoter haplotypes was analysed, and the results are shown in Table II. No significant differences in the clinical features between patients with various genotypes/haplotypes could be demonstrated. The association between the clinical manifestations of SLE patients with *TNF*-α genetic polymorphism was also investigated. The allele and genotype frequencies of the polymorphisms at -308 were significantly different in patients with serositis, oral ulcers, photosensitivity, discoid rash and malar rash (Table III). The G allele frequency was significantly decreased in patients with oral ulcers (*P*c=0.002).

IL-10 and TNF-α genotypes in Taiwanese SLE susceptibility and clinical features

SLE patients were also classified into the four possible combined genotypes to investigate potential *IL-10* and *TNF*-α interaction in SLE susceptibility (Table IV). Analysis of the combined *IL-10* and *TNF*-α genotypes yielded a significant different distribution in patients and controls ($p<0.001$, 4x2 contingency table). The combined frequencies of *IL-10* A/T/A haplotype and *TNF*-α G-G genotype were significantly increased in SLE patients (79.5% in SLE patients and 68.1% in controls; $p=0.012$), whereas the fre-

Supplemental Table I. IL-10 promoter polymorphisms evaluated in Taiwanese SLE patients and controls.

Gene name (Nucleotide change) (SNP database ID)	Report 1 Dye	Allele	Report 1 Quencher	Report 2 Dye	Allele	Report 2 Quencher	Context Sequence
IL-10 -1082 (A/G) (rs1800896)	VIC	T	NFQ	FAM	C	NFQ	TCCTCTTACCTATCCCTACTTCCCC[T/C]TCCCAAAGAAGCCTTAGTAGTGTTG
IL-10 -819 (C/T) (rs1800871)	VIC	A	NFQ	FAM	G	NFQ	AGTGAGCAAACTGAGGCACAGAGAT[A/G]TTACATCACCTGTACAAGGGTACAC
IL-10 -592 (C/A) (rs1800872)	VIC	T	NFQ	FAM	G	NFQ	CTTTCCAGAGACTGGCTTCCTACAG[T/G]ACAGGCGGGGTCACAGGATGTGTTC

The genotyping method was designed by TaqMan(R) Genotyping Assays (Applied Biosystems).

Supplemental Table II. TNF-α polymorphism evaluated in SLE susceptibility and related clinical features.

Gene name (Nucleotide change) (SNP database ID)	Primers	PCR product size (bp)	PCR conditions (annealing temperature)	Restriction enzyme site	Alleles	DNA fragment size (bp)	Position
TNF-α-308 (A/G) (rs1800629)	Forward:5'-AGGCAATAGGTTTTGAGGGCCAT-3' Reverse:5'-ACACTCCCCATCCTCCCGGCT-3'	117	ouchdown 60-50°C	Nco I	A G	117 97.20	chr6:31,651,010

quencies of *IL-10* non A/T/A haplotype and *TNF*-α G-G genotype were significantly decreased in SLE patients (2.5% in SLE patients and 14.3% in controls; $p<0.001$). The association between the clinical manifestations of SLE patients with the combined genotypes was also investigated (Table V). The combined frequencies of *IL-10* A/T/A haplotype and *TNF*-α G-G genotype were significantly different in patients with oral ulcers (Table V). The combined frequencies of *IL-10* A/T/A haplotype and *TNF*-α G-G genotype were significantly decreased in patients with oral ulcers ($Pc=0.004$).

Discussion

We have found a relation between polymorphisms at the promoter region of *IL-10* and *TNF*-α genes and the SLE susceptibility and a significant correlation of the combined *IL-10* and *TNF*-α genetic polymorphisms contribute to clinical features was also observed in the Taiwanese population.

Our results showed that *IL-10* A/T/A-A/T/A genotype was associated with Taiwanese SLE, whereas, no significant differences in the clinical features between patients with various genotypes/haplotypes could be demonstrated. Many studies have shown conflicting evidence for and against association of SLE with various *IL-10* genetic polymorphisms (13-17, 35-40). Rood *et al*. reported that the ATA haplotype is associated with neuropsychiatric manifestations of SLE among the Dutch population,(15) while Mok *et al*. indicated that this haplotype is not associated with SLE susceptibility, but is associated with renal involvement in the SLE patients (16). However, Chong *et al*. identified that the non-ATA haplotype is associated with SLE patients with serositis (17). These results suggest that SLE is a complicated disease. And the involvements of genetic factors as well as certain environmental factors might also be essential in disease predisposition and progression.

The allele and genotype frequencies of the polymorphism at -308 were not significantly different in Taiwanese SLE patients and controls. Many studies have also shown conflicting evidence for and against association of SLE with various *TNF* locus polymorphisms (25-31). A recent meta-analysis of the *TNF*-α -308G/A promoter polymorphism in SLE revealed a significant association found in European population, but not in Asian or African populations (27). Our results are in agreement with this conclusion that there is no significant association at the -308 position of *TNF*-α promoter gene in our Taiwanese population. However, the G allele of the polymorphism at -308 was significantly decreased in patients with oral ulcers. The functional analysis of polymorphism in the promoter region of *TNF*-α -308 position yielded conflicting observations. Some studies have suggested that the *TNF*-α -308 A allele has higher transcriptional activity, while other studies showed that this polymorphism appeared not to influence *TNF*-α production (41-44). The association between the genetic polymorphism at -308 and *TNF*-α production remains to be characterised.

Since the two regulators of the inflammatory response – *IL-10* and *TNF*-α have been suggested for the involvement of SLE pathogenesis, we have also investigated the interaction between *IL-10* and *TNF*-α in Taiwanese SLE susceptibility as well as clinical features. Our results showed that the combined frequencies of *IL-10* A/T/A haplotype and *TNF*-α G-G genotype were significantly increased in SLE patients. In addition, the combined frequencies of *IL-10* A/T/A haplotype and *TNF*-α G-G genotype were significantly decreased in patients with oral ulcers. The effects of cytokines may be greatly conditioned by the presence of other cytokines, particularly in the case of *IL-10* and *TNF*-α, which have complex and predominantly opposing roles in the systemic inflammatory responses. Suarez *et al*. reported that in Spanish SLE patients, there was a strong association between susceptibility to SLE and the high *TNF*-α producer genotype,

independently of IL-10 production and individuals harbouring high IL-10/low *TNF*-α producer genotype tend to develop DLE. These results suggest there may be ethnic difference, genetic factors as well as certain environmental factors involved in SLE disease predisposition and progression.

In conclusion, our observations suggest that the combined genotypes showed an association with *IL-10/ TNF*-α genotype in Taiwanese SLE patients and could influence different SLE phenotypes.

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