Jpn J Human Genet 42, 241-247, 1997

RFLP Report

*Nco*I RESTRICTION FRAGMENT LENGTH POLYMORPHISM AT −308 OF THE TUMOR NECROSIS FACTOR ALPHA (*TNFA*) PROMOTER REGION IN KOREAN

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Summary Tumor necrosis factor alpha (*TNFA*) is a cytokine, which is secreted from activated macrophage, with a broad range of biological activities. The gene encoding *TNFA* is located in tandem with the *TNFB* gene within the HLA complex on chromosome 6p21.3. We detected a single base polymorphism in the human *TNFA* gene promoter region in 300 unrelated Korean individuals. The *TNFA* promoter region which showed a G to A transition at position of −308 was investigated by *Nco*I restriction fragment length polymorphism analysis. A biallelic polymorphism of *TNFA* gene showed fragments of 87/20 bp and 107 bp acting as *TNFA*1* allele and *TNFA*2* allele, respectively. The allele frequencies of *TNFA*1* and *TNFA*2* were 0.8783 and 0.1217, respectively. The 21.7% of heterozygosity was observed. No association between promoter region phenotypes of *TNFA* and the first intron phenotypes of *TNFB* was observed in Korean. Allele frequencies of Koreans were compared with that of Europeans.

Key Words *TNFA*, *Nco*I-RFLP, Korean

Introduction

The gene encoding tumor necrosis factor alpha (*TNFA*) is located in tandem with the tumor necrosis factor beta (*TNFB*) gene between *HLA-B* and *C2* gene within the MHC region on chromosome 6p21.3. (Spies *et al.*, 1986; Carroll *et al.*, 1987; Ziegler *et al.*, 1991). The primary transcript of human TNF-α consists of 2,762 bp which has four exons and three introns (Nedwin *et al.*, 1985; Nedospasov *et al.*, 1986). TNF-α and TNF-β, which have similar biological activities, share approximately 30% amino acid homology (Goeddel *et al.*, 1986).

Received September 13, 1996; Revised version accepted November 14, 1996.
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TNF-α is a cytokine with tumoricidal and tumorstatic properties and an endogenous mediator of inflammatory functions *in vivo*, although the mechanism is not fully understood. The major sources of TNF-α are the activated macrophage, monocyte, and several other cell types (Sariban *et al.*, 1988; Aggarwal, 1992).

The DNA sequences of *TNFA* gene can vary in a normal population whether or not they code for a protein. Moreover, the DNA sequences determine a phenotype. A polymorphism in *TNFA* gene is determined not only by direct sequencing (Messer *et al.*, 1991) but also by other methods: *i.e.*, polymerase chain reaction *Nco*I restriction fragment length polymorphism (PCR-*Nco*I-RFLP) typing (Wilson *et al.*, 1992), polymerase chain reaction single strand conformational polymorphism typing (PCR-SSCP) (Wilson *et al.*, 1993), and polymerase chain reaction allele specific oligonucleotide typing (PCR-ASO) (D'Alfonso and Richiardi, 1994). We applied the PCR-*Nco*I-RFLP method to screen polymorphic variations at position −308 of the *TNFA* promoter in unrelated Korean individuals. Furthermore, the association between promoter region phenotypes of *TNFA* and the first intron phenotypes of *TNFB* was examined.

Materials and Methods

The subjects in the sample were comprised of 300 unrelated Koreans. Genomic DNA was prepared from peripheral blood by proteinase K (Boehringer-Mannheim) and phenol-chloroform extraction. Extracted genomic DNA (0.2 μg) was amplified by the polymerase chain reaction with 1 unit of the *Taq* DNA polymerase (Poscochem R&D Center) and 20 nmol of each primer in a Perkin/Elmer Cetus 9600. For *TNFA*, following primers were used: 5'AGGCAATAGGTTTTGAGGGCCAT3' (TNFA1), and 5'TCCTCCCTGCTCCGATTCCG3' (TNFA2) which were hybridized to positions of −332 to −310 and of −245 to −226 of the TNFA gene (Fig. 1) (Wilson *et al.*, 1992).

Reaction conditions contained 200 μM of dNTPs (Boehringer-Mannheim), 10× reaction buffer (500 mM KCl, 1.0% Triton X-100, 100 mM Tris-HCl, pH 9.0), 15 mM $MgCl_2$. Cycling was performed at 94°C for 4 min, 58°C for 20 sec, and 72°C for 35 sec followed by 35 cycles of 94°C for 30 sec, 58°C for 20 sec, and 72°C for 35 sec with final cycle of 94°C for 30 sec, 58°C for 20 sec, and 72°C for 4 min. The expected PCR products were separated on 10% polyacrylamide gel, stained with ethidium bromide (EtBr) and visualized under UV light. Amplified product of *TNFA* was digested with 1 unit of restriction enzyme, *Nco*I, for 12 hr at 37°C. To assign *Nco*I digestion of amplified *TNFA* gene, cleaved DNA was analyzed by electrophoresis using 10% polyacrylamide gel and stained with EtBr.

Results and Discussion

Three-hundred unrelated Korean subjects were screened for the polymorphism at −308 of *TNFA* promoter region. A 107 base pair sequence including the

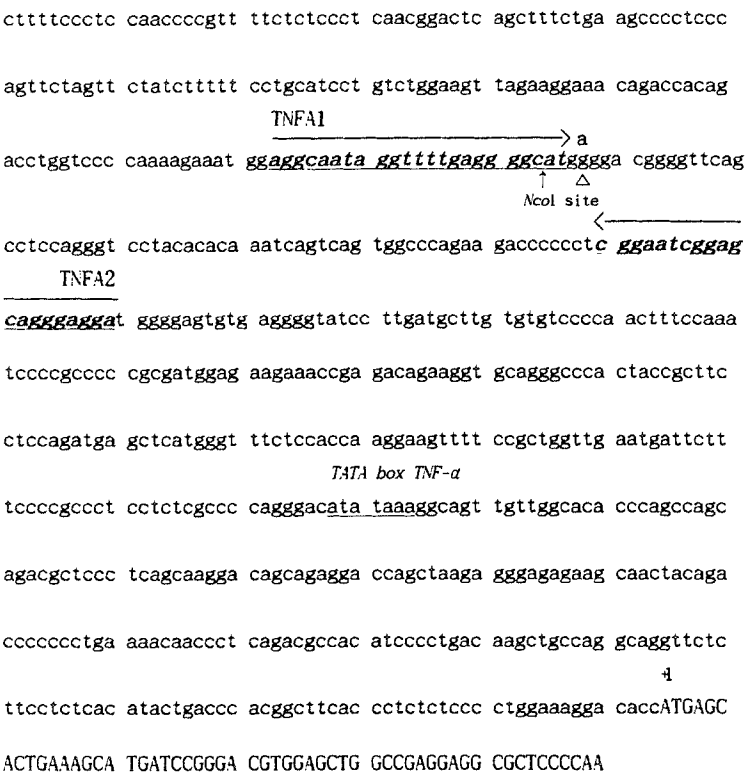

Fig. 1. Sequence of the 5′ promoter region of the *TNFA* gene (Nedospasov *et al.*, 1986). The sequence is numbered in relation to the transcription start site (+1). The TATA boxes are underlined and the arrowed lines are indicated as the two oligonucleotide primers for PCR amplification (from −332 to −226) (Wilson *et al.*, 1992). The polymorphism at position −308 is indicated by a triangle. The capital letters are exon regions.

polymorphic site in *TNFA* promoter region, from position of −332 to −226, was amplified and a polymorphism was observed in a G *vs* A transition at position −308 restriction site. Synthetic oligonucleotide primers were designed to recognize *Nco*I by a single base change at the 3′ end of primer TNFA1 and to yield the expected size fragment. While the *TNFA*1* allele corresponding to *Nco*I fragment of 87/20 bp showed the presence of CCATGG sequence, the *TNFA*2* allele corresponding to 107 bp fragment lacked *Nco*I restriction site because of a point mutation of G sequence to A sequence (Fig. 2).

Using SSCP, the two alleles were clearly identified after partial denaturation of the PCR product at the same position of −308 *TNFA* promoter region (Wilson *et al.*, 1993, 1994). Wilson *et al.* (1992) showed that the mendelian segregation of this polymorphism was observed in 5 members of one family spanning two

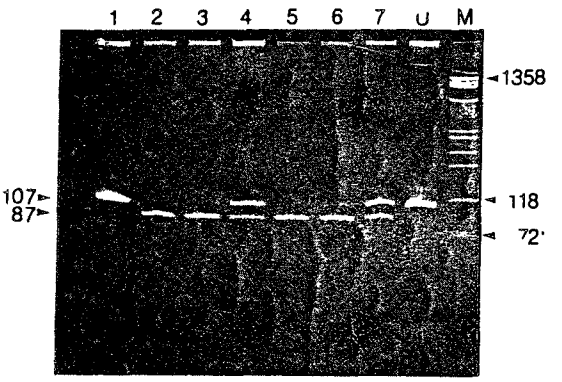

Fig. 2. Separation by 10% gel electrophoresis of the fragments obtained after PCR amplification of a 107 bp segment of the *TNFA* gene digestion with *Nco*I. *TNFA*1* (87+20 bp) and *TNFB*2* (107 bp) fragments indicate *Nco*I site positive and negative. M: the size marker ϕX174/*Hae*III; U: *Nco*I untreated; *TNFA*1/TNFA*1*: lanes 2, 3, 5, 6; *TNFA*1/TNFA*2*: lanes 4, 7; *TNFA*2/TNFA*2*: lane 1.

generations.

Recently the polymorphism of the human *TNFA* promoter at different region was reported (Messer *et al.*, 1991; D'Alfonso and Richiardi, 1994). The other biallelic polymorphism at position of −238 was observed including the substitution of guanine by adenosine using PCR-ASO (presence of A sequence= *TFNA-A* allele, presence of G sequence= *TNFA-G* allele) typing. The gene frequencies of *TNFA-A* and *TNFA-G* were 0.93 and 0.07, respectively (D'Alfonso and Richiardi, 1994). Messer and coworker (1991) showed a deletion of cytosine in *TNFA* promoter region (−687 to −370) compared with other sequences by PCR-directed sequencing. But this position of *TNFA* promoter region did not reveal any polymorphic sequence variation.

In this study, the frequencies of *TNFA* genotypes at −308 position in *TNFA*1/TNFA*1*, *TNFA*1/TNFA*2*, and *TNFA*2/TNFA*2* were 77% (n=231), 21.7% (n=65) and 1.3% (n=4), respectively. The 21.7% of heterozygosity was observed. The allele frequencies of *TNFA*1* and *TNFA*2* were 0.8783 and 0.1217, respectively. The test for Hardy-Weinberg equilibrium among random individuals showed no significant deviation from our expectations (Table 1).

The gene frequencies of *TNFA* in European and Korean populations are shown in Table 2. *TNFA*1/TNFA*1* is common phenotype, and the *TNFA*1* allele frequencies of Koreans are higher relative to that of the Europeans (Wilson *et al.*, 1994; Cox *et al.*, 1994; Brinkman *et al.*, 1994).

It was suggested that *TNFA* expression depends on polymorphic variations of *TNFA* promoter region itself or linkage association with HLA genotype (Molvig

Table 1. Distribution of genotype at position −308 of the *TNFA* promoter region and allele frequencies by *Nco*I in Korean.

Genotype	Obs. (%)	Exp. (%)	Allele frequencies±SE
*TNFA*1/TNFA*1*	231 (77.0)	231.4 (77.1)	*TNFA*1*=0.8783±0.0133
*TNFA*1/TNFA*2*	65 (21.7)	64.2 (21.4)	*TNFA*2*=0.1217±0.0133
*TNFA*2/TNFA*2*	4 (1.3)	4.4 (1.5)	
Total	300 (100.0)	300.0 (100.0)	

$\chi^2=0.05$, d.f.=1.

Table 2. Comparison of allele frequencies at position −308 of the *TNFA* promoter region by *Nco*I RFLP.

Population	n	*TNFA*1*	*TNFA*2*	References
Korean	300	0.88	0.12	This study
European	168	0.83	0.17	Wilson *et al.*, 1994
England	253	0.78	0.22	Cox *et al.*, 1994
Netherlands	88	0.76	0.24	Brinkman *et al.*, 1994

Table 3. Association analysis between phenotypes at position −308 of *TNFA* promoter region and the first intron region phenotypes of *TNFB* in Korean.

TNFA	*TNFB*			
	1-1	1-2	2-2	Total
1-1	23 (7.7%)	103 (34.3%)	105 (35.0%)	231 (77.0%)
1-2	3 (1.0%)	33 (11.0%)	29 (9.7%)	65 (21.7%)
2-2	0 (0.0%)	1 (0.3%)	3 (1.0%)	4 (1.3%)
Total	26 (8.7%)	137 (45.6%)	137 (45.7%)	300 (100.0%)

et al., 1988; Han *et al.*, 1990, 1991; Wilson *et al.*, 1993; D'Alfonso and Richiardi, 1994). Wilson *et al.* (1993) postulated that *TNFA* promoter region to be important in regulation of transcription and described a very strong association between the *TNFA*2* allele and HLA A1, B8, DR3 and DQ2 haplotype. Furthermore, Wilson *et al.* (1994) investigated the significance of *TNFA* polymorphism in relation to autoimmune disorder where genetic factors play a role in susceptibility and autoantibody production. The frequency of the *TNFA*2* allele increased in systemic lupus erythematosus compared with controls ($p=0.04$).

We previously studied an *Nco*I restriction site polymorphism in the first intron of the *TNFB* gene and examined an association between promoter region phenotypes of *TNFA* and the first intron phenotypes of *TNFB* was analyzed in 300 unrelated Korean individuals. We observed no association between *TNFA* and *TNFB* alleles (Table 3), and this finding did not correspond to the results of Netherlands reported by Brinkman *et al.* (1994).

The polymorphism screened in this study was characterized in the *TNFA* gene of Korean population.

Acknowledgments The present study was supported by a grant from the Korea Science and Engineering Foundation, KOSEF 95-0403-64-3.

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