CTLA-4 dimorphisms in Japanese patients with systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of various autoantibodies. Genetic predisposition has been implicated in the pathogenesis of SLE in various studies, though the etiology of SLE is still unclear.

Cytotoxic T lymphocyte associated-4 (CTLA-4) and CD28 on T cells, on the other hand, bind CD80 and CD86, and CTLA-4 is a negative regulator of T cell activation (1). The CTLA-4 gene is located on chromosome 2q33 and the two dimorphisms, +49A/G and -318C/T, have been reported in exon 1 and in the promoter region, respectively (2, 3). Associations between these two CTLA-4 dimorphisms and autoimmune diseases including SLE have been reported by several groups (2, 4, 5), despite the absence of any associations with SLE in other reports (6-8).

Considering the immune-regulatory function of CTLA-4, the CTLA-4 gene is an interesting candidates as a disease-susceptible gene or genetic marker. We studied Japanese SLE patients to clarify the contribution of CTLA-4 genes to the disease using the PCR-RFLP method (2, 3).

Forty-seven randomly selected unrelated Japanese SLE patients (aged 38.0 ± 12.9; 45 women and 2 men) diagnosed according to the 1982 criteria laid out by the American Rheumatism Association were examined. The dimorphisms at position +49 (+49A/G) and at position -318 (-318C/T) were detected by the PCR-RFLP method using specific oligonucleotide primers described previously (2, 3, 9). Fisher's exact test was used for comparison of genotype and allele frequencies (9). HLA-DRB1*1501, the susceptible gene reported, was genotyped by the PCR-SSCP (single-stranded DNA conformation polymorphism) method described previously (9). Anti-nuclear antibody (ANA), anti-SS-A, anti-SS-B, anti-U1-RNP, anti-Scl-70 and anti-double-stranded DNA autoantibodies (a-SS-A, a-SS-B, a-RNP, a-Sm, a-Scl-70 and a-DNA, respectively) were detected by the standard method in the Central Laboratory Service of Tokyo University Hospital, described previously (9).

Genotype and allele frequencies of the +49A/G dimorphism are shown in Table I. No CTLA-4 +49A/G genotype or allele was found to be significantly associated with SLE. Genotype and allele frequencies of the -318C/T dimorphism are shown in Table I. In the SLE group, no patients showed -318T/T genotypes, but no significant difference in allele frequency was observed, either.

The association between the CTLA-4 dimorphisms and autoantibodies are shown in Table I. The +49AG genotype slightly increased (52.9%) and the +49AA genotype decreased (5.9%) in the a-RNP positive group, though these differences were not significant. The genotypes +49AA and +49AG slightly increased (18.8% and 50.0%, respectively) and the GG genotype decreased (31.3%) in the a-SS-A positive group but, again, the increase was not significant,either. The genotype frequency of -318CC slightly increased in the a-RNP positive and a-SSA positive groups (82.4% and 81.3%, respectively), though these differences were not significant. No significant association of the +49A/G and the -318C/T dimorphism with autoantibodies examined, was observed, as well.

The association of CTLA-4 with SLE having HLA-DRB1*1501, that is reported as a susceptibility gene in Japanese, was examined because the possibility that CTLA-4 co-contributed to the pathogenesis of SLE together with HLA-DR still remained (10). The genotype frequency of the AA (+49A/G) did not significantly increase in HLA-DRB1*1501 positive SLE patients (42.9%) (Table I). The slight increase in the allele frequency of the +49A allele was not significant, either. There was no significant association between the -318 dimorphism and SLE patients positive for HLA-DRB1*1501.

In this experiment no association of CTLA-4 dimorphisms (+49A/G and -318C/T) was observed, though Ahmed *et al.* recently reported (5) the significant increase of the CTLA-4 +49G allele in Japanese SLE patients. An association study of CTLA-4 with autoantibodies showed no significant association either, suggesting strongly that CTLA-4 dimorphisms are not involved in the autoantibodies in Japanese SLE patients. The synergistic effect of CTLA-4 RFLP on the SLE-susceptible HLA-DR allele, DRB1*1501, was not observed in Japanese SLE, also. The finding supports the previous report of D'Alfonso *et al.* (6), regarding Italian SLE patients.

Our observation showed no association of CTLA-4 dimorphisms (+49A/G and -318C/T) with Japanese SLE. It is very likely that the CTLA-4 gene is not genetically involved in the pathogenesis of Japanese SLE.

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Table I. Association of CTLA-4 dimorphisms (+49A/G & -318C/T) with SLE.

	Normal	whole SLE	a-DNA+	a-RNP+	a-Sm+	a-SSA+	1501+
N	107	47	33	17	4	16	7
+49A/G Genotype							
AA	13 (12.1%)	7 (14.9)	4 (12.1)	1 (5.9)	1 (25.0)	3 (18.8)	3 (42.9)
AG	45 (42.1)	18 (38.3)	14 (42.5)	9 (52.9)	1 (25.0)	8 (50.0)	1 (14.3)
GG	49 (45.8)	22 (46.8)	15 (45.5)	7 (41.2)	2 (50.0)	5 (31.3)	3 (42.9)
+49A/G Allele							
A	71 (33.2)	32 (34.0)	22 (33.3)	11 (32.4)	3 (37.5)	14 (43.8)	7 (50.0)
G	143 (66.8)	62 (66.0)	44 (66.7)	23 (67.6)	5 (62.5)	18 (56.3)	7 (50.0)
-318C/T Genotype							
CC	83 (77.6)	38 (80.9)	26 (78.8)	14 (82.4)	3 (75.0)	13 (81.3)	5 (71.4)
CT	21 (19.6)	9 (19.1)	7 (21.2)	3 (17.6)	1 (25.0)	3 (18.8)	2 (28.6)
TT	3 (2.8)	0	0	0	0	0	0
-318C/T Allele							
C	187 (87.4)	85 (90.4)	59 (89.4)	31 (91.2)	7 (87.5)	29 (90.6)	12 (85.7)
T	27 (12.6)	9 (9.6)	7 (10.6)	3 (8.8)	1 (12.5)	3 (9.4)	2 (14.3)