

BRIEF COMMUNICATION

A CTLA-4 gene polymorphism at position –318 in the promoter region affects the expression of protein

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CTLA-4 is an important negative regulator of the immune system. The regulation of the CTLA-4 gene (Ctla-4) transcription is poorly understood. A single nucleotide polymorphism (SNP) at –318 in the Ctla-4 promoter region is associated with certain autoimmune diseases. Since the –318 SNP occurs in a potential regulatory region, it is conceivable that the C' T transition may affect the expression of Ctla-4. In the present study, we show that the –318T allele is associated with a higher promoter activity than the –318C allele (8.13 ± 0.46 vs 6.87 ± 0.49). The presence of the –318T allele may thus contribute to up regulation of the expression of CTLA-4, and consequently represent one mechanism to inhibit exaggerated immune activity.

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CTLA-4 plays an important role in the immune system as a T cell inhibitory factor. This inhibitory signal not only determines whether T cells become activated, but also affects the clonal representation in an immune response. CTLA-4 regulates cell cycle progression rather than directly the induction of apoptosis.¹ CTLA-4 Ig and anti-CTLA-4 have been applied for the treatment of both autoimmune diseases and cancers.

Little is known about the regulatory mechanism for CTLA-4 expression. Polymorphisms in the *Ctla-4* are associated with several autoimmune diseases, and polymorphisms at certain locations of the *Ctla-4* might conceivably affect its expression. We have described a higher prevalence of thymine at position –318 of the *Ctla-4* promoter (–318T) in patients with Wegener's granulomatosis.² Recently, Ligiers and colleagues reported that individuals carrying –318T exhibited significantly increased expression both of cell-surface CTLA-4 in activated cells and of *Ctla-4* mRNA in non-stimulated cells,³ suggesting a potential role for the C' T transition in the regulation of expression. In the present study, we examined the effect of C' T switch at –318 on the *Ctla-4* promoter activity. Our results demonstrated that the –318T allele was associated with a significantly higher promoter activity than the –318C allele.

Results and discussion

To determine whether the C' T transition at position –318 transcriptionally regulates the *Ctla-4* gene expression, we made two *Ctla-4* promoter reporter constructs which contained C and T alleles, respectively. As shown in Figure 1, the –318T allele was associated with a higher promoter activity than the –318C allele (8.13 ± 0.46 vs 6.87 ± 0.49). This result was also confirmed in the THP-1 cell line transfected with these two reporter constructs, and stimulated with IFN- γ (5 mg/ml) and PMA (10 ng/ml).

Our allele-specific transcription data indicate that the –318T allele is associated with higher transcriptional activity than the –318C allele. The –318T allele could be considered as protective against autoimmune reactions. Our results could explain the increased expression of CTLA-4³ and the observed associations of the –318T allele to some diseases.⁴

Homozygosity for –318/T in the promoter of *Ctla-4* is rarely present in a Caucasian population.^{4–7} In our previous study, the frequencies of individuals with genotypes –318C/C, C/T, and T/T were 86%, 17% and 0%, respectively, in healthy individuals ($n = 122$) and 69%, 31%, 0%, respectively, in patients with Wegener's granulomatosis ($n = 32$). When we considered the prevalence of longer (AT) n in the 3'-untranslated region together with the promoter single nucleotide polymorphism (SNP), we found that all patients carrying the –318T allele were homozygous for (AT) $n > 86$ bp alleles.² As the longer (AT) n alleles are associated with decreased mRNA stability and thus decreased protein expression, the –318T mutation should counteract the effects of long (AT) n alleles. The –318T mutation could thus be regarded as protective against increased T cell stimulation.

In conclusion, the C' T transition at position –318 transcriptionally regulates the *Ctla-4* gene expression and the

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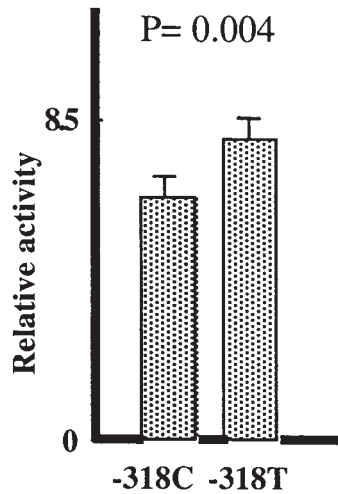


Figure 1 Relative promoter activity of the human *Ctla-4* gene in Jurkat T cells. Genomic DNA was used to amplify a 329-bp fragment covering the -318 polymorphic site. The following primers were used: -318C forward: AGG GGT ACC ACT TAG TTA TCC AGA TCC TCA AAG, -318T forward: AGG GGT ACC ACT TAG TTA TCC AGA TCC TTA AAG, and reverse: AG CGC TCG AGC CAG GTT CAG. The amplified fragments (318C and -318T alleles) were subcloned into a TA cloning vector (PCRII; In Vitrogen, Leek, The Netherlands). The sequence of inserted fragment was confirmed by sequence analysis using the ABI-PRISM Terminator Cycle Ready Reaction Kit (Perkin Elmer, Foster City, CA, USA). Each fragment was then inserted upstream of the firefly luciferase reporter gene at the *KpnI/XhoI* sites of the PGL3-basic vector (Promega, Madison, WI, USA). Jurkat T cells were maintained in complete medium (CM) (RPMI1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin) at 37°C under 5% CO₂. Transfection experiments were conducted using the FuGENE 6 Transfection Reagent from Roche Molecular Biochemical (Stockholm, Sweden). Twenty-five thousand cells were seeded with 1 mg of constructs and 100 ng of pRL.CMV (a *Renilla*-based luciferase construct, Promega) per-well. After 24 h, the cells were stimulated with ConA (5 mg/ml), PMA (10 ng/ml) and harvested after 48 h. Dual-Luciferase Report assay system (Promega) was applied according to the manufacturer's instructions. The luciferase activity was measured using a Turner luminometer (TD-20/20). Firefly luciferase activity was normalized to *Renilla* luciferase activity to correct for variations in transfection efficiency. The relative luciferase levels were obtained from three assays performed in duplicate.

T mutation could be considered as protective for autoimmune disease.

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