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Analysis of linkage between lymphotoxin α haplotype and polymorphisms in 5′-flanking region of tumor necrosis factor α gene associated with efficacy of infliximab for Crohn's disease patients

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Abstract

Tumor necrosis factor (TNF)α is increased in patients with Crohn's disease (CD) and considered to play an important role in the inflammation. Infliximab (IFX) is used as a therapeutic agent for CD. Recently, it was reported that homozygosity for a lymphotoxin α (*LTA*) haplotype (*LTA* 1-1-1-1) may identify subgroups with a poor response to IFX. In the present study, we characterized the linkage of the *LTA* haplotype with SNPs in the 5′-flanking region of the *TNFα* gene. In subjects who had homozygosity for each *LTA* haplotype, 6 nucleotide variations, −857C > T, −522C > G, −357A > C, −261C > G, −159G > T and −96G > T, were found in the 5′-flanking region of the *TNFα* gene. As for linking with the allele, only −857T met the *LTA* haplotype 1-1-1-1. We concluded that the differences in therapeutic effects of IFX among patients with CD may be explained in part by the induction ability of TNFα via the −857C > T polymorphism.

Keywords: Crohn's disease; Infliximab; Tumor necrosis factor α; Lymphotoxin α; Haplotype analysis

1. Introduction

Crohn's disease (CD) is characterized by chronic transmural inflammation in any part of the gastrointestinal tract [1]. The major symptoms are diarrhea, abdominal pain, fistulas, and weight loss, and the peak age at onset ranges from 15 to 25 years old. Tumor necrosis factor (TNF)α, a primary mediator of inflammatory responses, is increased in patients with active CD [2] and considered to play an important role in the regulation of inflammation in those patients. Infliximab (IFX) is a chimeric murine-human monoclonal IgG_1 antibody that targets TNFα and used as a therapeutic agent for CD, as it binds with a high affinity to TNFα and neutralizes its effects [3].

Although most patients treated with IFX show a clinical response, for some the effects are insufficient [4–6], while that therapy can also cause serious side effects, such as severe infections and tuberculosis. Therefore,

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it is important to understand the possible therapeutic effects of IFX for CD patients prior to treatment. Thus far, various factors including genetic and environmental factors, as well as biomarkers, have been examined to elucidate the activities of IFX in different patients. Of those, it has been speculated that quantitative and qualitative changes of TNFα are important in regards to the therapeutic effect for patients with CD. Some genetic-polymorphisms in the *TNFα* gene have been reported and single nucleotide polymorphisms (SNPs) in the 5′-flanking region of the *TNFα* gene, −238G > A, −308G > A, −857C > T, −863C > A and −1031T > C, are known [7–10]. In addition, TNFa, TNFb, TNFc, TNFd and TNFe, microsatellites of the *TNFα* gene and lymphotoxin α (*LTA*) gene, which is in the neighborhood of the *TNFα* gene, have been reported [11]. Although some have studied the relationship between −308G > A and the therapeutic effect of IFX, other results are scarce [12].

Recently, homozygosity for the *LTA Nco*I-TNFc-aa13L-aa26 haplotype 1-1-1-1, which exists near the *TNFα* gene and *LTA* gene loci, has been shown to identify subgroups with a poor response to IFX [13]. The involvement of TNFα with CD clinical conditions and the therapeutic effect of IFX has been suggested, therefore, we speculated that an SNP in the 5′-flanking region of the *TNFα* gene linked to the *LTA* haplotype is responsible for inter-individual differences in the expression of TNFα. In the present study, we investigated the linkage between the *LTA Nco*I-TNFc-aa13L-aa26 haplotype and SNPs in the 5′-flanking region of the *TNFα* gene.

2. Materials and methods

2.1. Subjects

To investigate the *LTA* haplotype and the sequence of the 5′-flanking region of *TNFα* gene, we used genomic DNA extracted from EB virus-immortalized B-cells derived from 98 Japanese individuals established by the Pharma SNP Consortium (PSC) [14]. We obtained the subjects from The Health Science Research Resources Bank (HSRRB). The use of the subjects in the present study was approved by the genome ethics committee of the HSRRB and by that of the PSC.

2.2. Genotyping

2.2.1. LTA locus

The oligonucleotide sequences of the primers used for PCR and sequencing are shown in Table 1. The *LTA* locus (*LTA Nco*I-TNFc-aa13L-aa26) was amplified in a reaction mixture (50 μl) containing ExTaq buffer (with 2.0 mM of magnesium chloride, TaKaRa), 10 pmol of each primer, 0.2 mM of dNTPs (Applied Biosystems), 0.8 units of Ex Taq HS DNA polymerase (TaKaRa), and 50 ng of genomic DNA as a template. PCR amplifications were performed as follows: 40 cycles with melting at 95 °C for 1 min, annealing at 62 °C for 1 min and extension at 72 °C for 1 min, after preheating at 95 °C for 2 min. The PCR product was digested with *Nco*I before electrophoresis on a 2% agarose gel. The *LTA Nco*I allele (type 1) did not possess an *Nco*I restriction site and gave a single 968-bp band, while another *LTA Nco*I allele (type 2) was digested with *Nco*I and gave 771- and 197-bp bands. In addition, the genotypes of *LTA* TNFc, aa13L and aa26, were determined by sequencing using the primers shown in Table 1. For the *LTA* TNFc microsatellite, the allele was distinguished by the number of TC repeats, as the *LTA* TNFc allele (type 1) was $(TC)_9$, while type 2 was $(TC)_{10}$. As for the genotyping of *LTA* aa13L, the 13th codon of the *LTA* gene encodes a cysteine in the type 1 allele (5′-CCA AGG GTG **TGT** GGC ACC-3′) and an arginine in the type 2 allele (5′-CCA AGG GTG **CGT** GGC ACC-3′). As for the genotype of *LTA* aa26, the 26th codon of the *LTA* gene encodes a threonine in the type 1 allele (5′-CCC CAC AGC **ACC** CTC AAA-3′) and an asparagine in the type 2 allele (5′-CCC CAC AGC **AAC** CTC AAA-3′). Based on the genotyping results, the combination of the *LTA Nco*I-TNFc-aa13L-aa26 haplotype of each subject was estimated using the HAPLOTYPER program [15].

Table 1
Sequences of primers used for genotyping

LTA gene [13]	
Forward	5′-CCCGTGCTTCGTGCTTTGG-3′
Reverse	5′-GAGTTCTGCTTGCTGGGGTCTCCT-3′
LTA gene sequencing	5′-GCATCTTGTCCCCTTCTCTG-3′
	5′-GGGTAGGAGGAGAGCTGGTG-3′
TNFα gene (Part A)	
Forward	5′-TGTCCAGGGCTATGGAAGTC-3′
Reverse	5′-GGACCAGGTCTGTGGTCTGT-3′
TNFα gene (Part B)	
Forward	5′-CTCCGGGTCAGAATGAAAGA-3′
Reverse	5′-AACAAGCACCGCCTGGAG-3′

2.2.2. TNFα gene

The 5′-flanking region of the *TNFα* gene was divided into 2 overlapping parts (Part A, 554 bp; Part B, 946 bp), which were amplified by PCR in reaction mixtures (50 μl) consisting of 1× PCR buffer II, 0.2 mM of dNTPs, 0.5 mM of each primer, 1.5 mM of magnesium chloride, 1 unit of Ampli Taq DNA polymerase (Applied Biosystems), and 50 ng of genomic DNA as a template. The conditions for PCR amplification were as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 10 min. The sequences of each PCR product were determined by sequencing, then the combination of the haplotype of the 5′-flanking region of the *TNFα* gene of each subject was estimated using the HAPLOTYPER program.