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Association between the Promoter Polymorphism T/C at Position -159 of the CD14 Gene and Anti-inflammatory Therapy in Patients with Inflammatory Bowel Disease

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Abstract: Immune response to intestinal bacteria and genetic predisposition seem to play a crucial role in the pathogenesis of inflammatory bowel disease. A single nucleotide polymorphism in the promoter of the lipopolysaccharide-receptor CD14 gene (T/C at position -159) has recently been described.

To evaluate the role of the CD14 gene in anti-inflammatory therapy, the functionally relevant T(-159)→C promoter polymorphism has been genotyped in 72 patients with inflammatory bowel disease and associated with the cumulative steroid dose.

Cumulative corticosteroid dose was significantly higher in ulcerative colitis patients with the TT genotype (2447.7 ± 927.0 mg/yr) compared with the CT genotype (142.3 ± 142.3 mg/yr, p=0.016) and the CC genotype (391.7 ± 272.7 mg/yr, p=0.047). In contrast, in patients with Crohn's disease there was no significant difference of the cumulative corticosteroid doses between the various T(-159)→C promoter CD14 genotypes.

An altered immune response to lipopolysaccharides with influence on the anti-inflammatory therapy seems to play a role in the genetic predisposition to ulcerative colitis. Genetic stratification will lead to the development of individualized therapies in inflammatory bowel disease.

Key words: Inflammatory bowel disease; CD14 gene

Abbreviations:

UC	Ulcerative Colitis
LPS	Lipopolysaccharides
LBP	LPS Binding Protein
SNP	Single Nucleotide Polymorphism
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
TLR	Toll-Like Receptor
NFκB	Nuclear Factor κB

Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are inflammatory bowel diseases (IBD) characterized by destructive inflammation of the intestinal wall. The initial trigger of the inflammatory response has not been identified so far, but there is increasing evidence both, from animal models and clinical investigations, that luminal bacteria play an important role in the pathogenesis of IBD. In addition, genetic predisposition to IBD is well established through epidemiological studies and genome wide linkage analyses and seems to have a crucial role in the aetiology of CD and UC [1-3].

Lipopolysaccharides (LPS) are the main endotoxins derived from gram-negative bacteria. LPS are bound with high affinity to LPS binding protein (LBP) [4]. The LPS/LBP complex interacts with the LPS receptor CD14 primarily expressed on inflammatory cells [5]. A single nucleotide polymorphism (SNP) in the promoter of the CD14 gene (T/C at position -159) has recently been described [6]. In addition, an association of this promoter SNP in the CD14 gene with CD has been demonstrated [7]. In order to evaluate whether this polymorphism contributes to the predisposition to the amount of anti-inflammatory therapy, we compared the cumulative dose of corticosteroid with the allele frequencies for this SNP.

Material and Methods

Blood Samples and DNA isolation

Blood samples of 72 consecutive patients with IBD were investigated (CD: n = 37, 20 women, 17 men, mean age 40.8 ± 11.5 yrs; UC: n = 35, 18 women, 17 men, mean age 46.4 ± 16.1 yrs). Diagnoses of IBD were confirmed by clinical, endoscopical and histological parameters according to the diagnostic criteria of the European Community Workshop on IBD [8]. DNA was isolated from EDTA-anticoagulated blood after standard protocols [9].

PCR

Primers CD14 Pro-F (5'-gtgccaacagatgaggttcac-3') and CD14 Pro-R (5'-gcctctgacagtttatgtaatc-3') were used to amplify a 497 bp fragment of the CD14 promoter. A

10 μL PCR amplification mixture containing 50 ng DNA, 1 U Taq polymerase (Genecraft), 0.2 mmol of each nucleotide, 1 mmol MgCl2 in 500 mmol KCl and 100 mmol Tris-HCl (pH 8.3) was run in a Thermocycler (Trio-Block, Biometra). After two initial cycles at 62 °C and 59 °C annealing temperature, 25 cycles at 95 °C, 56 °C annealing temperature and 72 °C were run 0.5, 1 and 1 min, respectively.

RFLP Analysis

The T allele of the polymorphism at position -159 contains the restriction site for Ava II, which is not present in the C allele. The PCR was restricted with Ava II and the resulting fragments (497 bp for the C; 353 bp, and 144 bp for the T allele, respectively) were separated on 2 % agarose gels and stained with ethidium bromide.

Cumulative Steroid Dose and Statistics

Cumulative corticosteroid dose of each patient over a period of 3 years was determined using standardized IBD questionnaire. Patients received no other immunosuppressive therapy. Results were analysed by Mann-Whitney-U-testing.

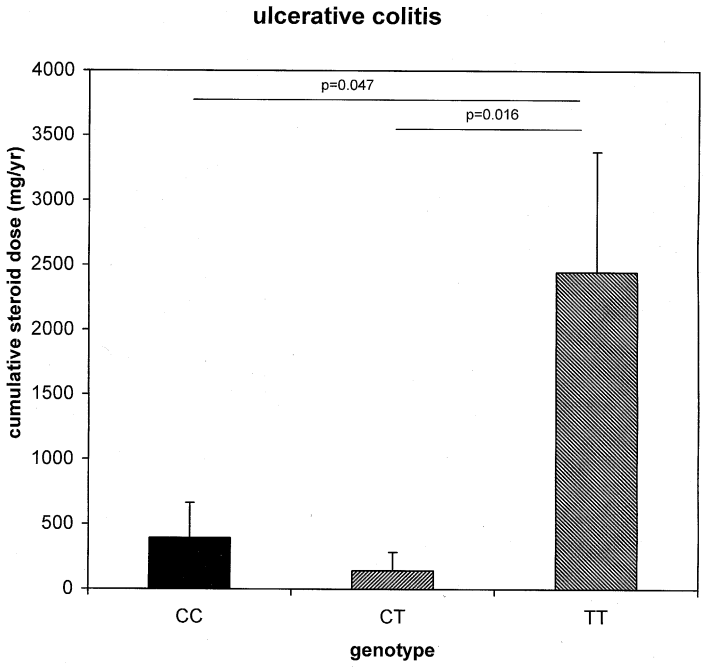

Fig. 1. Cumulative steroid doses in comparison to the genotypes of the promoter polymorphism T/C at position -159 of the CD14 gene in patients with ulcerative colitis.

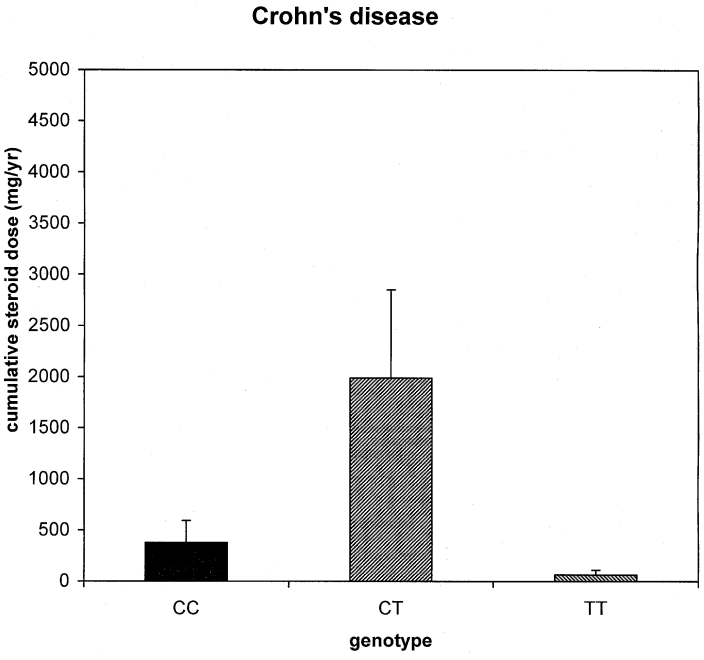

Fig. 2. Cumulative steroid doses compared with the genotypes of the promoter polymorphism T/C at position -159 of the CD14 gene in patients with Crohn's disease. Differences between the genotypes are not significant.

RESULTS

We have genotyped the promoter SNP (T/C at position -159) of the CD14 gene in 37 patients with CD and 35 patients with UC. Cumulative corticosteroid dose was significantly higher in UC patients with the TT genotype (2447.7 ± 927.0 mg/yr, n = 10) compared with the CT genotype (142.3 ± 142.3 mg/yr, n = 13, p=0.016) and the CC genotype (391.7 ± 272.7 mg/yr, n=9, p=0.047) (Fig. 1). In contrast, in patients with CD there was no significant difference of the cumulative corticosteroid doses between patients with the SNP (T/C at position -159) CD14 genotypes (TT: 61.0 ± 45.3 mg/yr, n = 9; CT: 1993.0 ± 858.7 mg/yr, n = 18; CC: 377.7 ± 214.7 mg/yr, n = 10) (Fig. 2).

DISCUSSION

The role of CD14 as a putative modifying gene for the cumulative steroid doses required in IBD patients was evaluated by genotyping an SNP in the promoter (T/C at position -159). CD 14 is a glycosyl-phosphatidylinositol-linked glycoprotein primarily expressed on the surface of monocytes, neutrophils and macrophages [5]. Elevated expression of CD14 by lamina propria mononuclear cells has been found in inflamed intestine of IBD patients [10, 11]. A soluble form of this receptor is found in high amounts in the serum. TT homozygotes have significantly higher serum levels of the soluble form of CD14 and lower levels of IgE [12]. The T allele is associated with increased risk of myocardial infarction [6]. LPS, as the main endotoxins derived from gram-negative bacteria, interact with the LPS receptor CD14 via an LPS/LBP complex. The signal transduction of the LPS/LBP/CD14 ternary complex is mediated by the toll like receptor 4 (TLR4) and results in activation of nuclear factor κB (NF κB) with subsequent production of proinflammatory cytokines such as IL-1, IL-6 and INF-γ [13-16]. Increased levels of these cytokines and NFκB are found in IBD [17-19].

In the present study, significantly higher cumulative corticosteroid doses as an indicator of the amount of anti-inflammatory therapy have been observed in TT homozygotes with UC. In contrast, there was no significant difference between the various genotypes in CD patients, may be due to the low number of patients. Our results indicate that the T allele and the TT genotype frequencies of the promoter polymorphism T/C at position -159 of the CD14 gene may be of evidence for the anti-inflammatory therapy of patients with UC. Polymorphisms in the abovementioned and further genes involved in regulation of cell-mediated immunity lead to qualitative or quantitative alterations of the encoded proteins thus restricting the capability of inflammatory cells to antagonize noxious bacteria. Abundant polymorphisms have been identified nearby these genes. However, these data are only suggestive and preliminary and thus have to be validated in further studies including examination of functional aspects like investigation of the therapeutic consequences as demonstrated in our study. Identification of further genes still remains an enormous task, because the role of a particular predisposing allele may only be relevant in a small fraction of patients, a specific population or in combination with other predisposing alleles [20,21]. Examination of these predisposing genes will lead to therapeutic stratification of patients with IBD.

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