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Interleukin 10 gene single nucleotide polymorphisms in Polish patients with chronic hepatitis C: Analysis of association with severity of disease and treatment outcome

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ABSTRACT

It is suggested that interleukin 10 (IL-10), as a modulator of immune response, is likely to influence the elimination of hepatitis C virus (HCV), the progression of chronic hepatitis C (CHC) and the response to interferon-based therapy in CHC patients. The aim of the study was to analyze the association of single nucleotide polymorphisms (SNPs) of *IL-10* gene with severity of liver disease (degree of inflammation and stage of fibrosis) and outcome of pegylated interferon alpha and ribavirin combined therapy (sustained virological response (SVR) and relapse) in 196 Polish CHC patients infected with HCV genotype 1. The analysis included *IL-10* promoter SNPs: −1082(A/G) rs1800896, −819(C/T) rs1800871, −592(C/A) rs1800872 and SNP in the 3′ UTR of *IL-10* gene: +4529(A/G) rs3024498. Genotyping was performed using PCR-RFLP and HRM analysis. It was demonstrated that the −592C allele is associated with mild hepatic inflammation. Moreover, it was found that the −819C allele might be associated with SVR and that the ACCA haplotype and intermediate *IL-10* producer ACC haplotype are associated with SVR and non-relapse. It can be concluded that *IL-10* SNPs are associated with severity of disease and response to therapy and may be considered as potential prognostic and predictive markers in CHC.

1. Introduction

Hepatitis C virus (HCV) infection is one of the leading cause of chronic liver disease and is considered as a major public health problem. It is estimated that HCV infects approximately 130–170 million people worldwide [1], whereas in Poland, the estimated number of people infected is about 730.000 [2]. Spontaneous viral clearance occurs in minority of cases, while 75–85% of infected individuals develop chronic infection (chronic hepatitis C, CHC). CHC is considered as a major cause of liver fibrosis. 10–15% of HCV-infected patients progress to liver cirrhosis and 1–4% of them develop hepatocellular carcinoma [3]. The treatment of CHC with pegylated interferon alpha (PEG-IFN-α) and ribavirin (RBV) is ineffective. Only about 50% of HCV genotype 1 patients

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHC, chronic hepatitis C; CI, confidence interval; EDTA, ethylenediaminetetraacetic acid; ETR, end of treatment response; G, grading; HCV, hepatitis C virus; HRM, high resolution melting; IFN-γ, interferon gamma; IL-10, interleukin 10; IL-2, interleukin 2; IL-28B, interleukin 28B; OR, odds ratio; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PEG-IFN-α, pegylated interferon alpha; RBV, ribavirin; RFLP, restriction fragment length polymorphism; S, staging; SNP, single nucleotide polymorphism; STAT1, signal transducer and activator of transcription 1; SVR, sustained virological response; TBIL, total bilirubin; Th1, Type 1 T helper; Th2, Type 2 T helper; TNF-α, tumor necrosis factor alpha.

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achieve sustained virological response (SVR), defined as undetectable serum HCV RNA six month after completion of therapy [4]. Moreover, about one-third of CHC patients with HCV genotype 1, who complete two-drug treatment and have undetectable serum HCV RNA at the end of treatment (end of treatment response, ETR) experience virological relapse [5]. The diversity regarding course of HCV infection and outcomes of anti-HCV therapy among individuals may be caused by host (age, gender, ethnicity), viral (HCV genotype, viral load) and environmental factors [6]. Genetic factors, which can affect an immune response, also appear to be crucial [7].

Interleukin 10 (IL-10) is a Th2-type anti-inflammatory cytokine, which acts in highly coordinated network and plays a crucial role in regulation of immune responses. This cytokine interferes with the balance of Th1/Th2 cells, negatively regulating the response of Th1 lymphocytes and suppressing the action of pro-inflammatory cytokines (such as interleukin 2, IL-2; tumor necrosis factor alpha, TNF-α; interferon gamma, IFN-γ) [8]. In infectious diseases, IL-10 is involved with bacteria and virus persistence by interfering with innate and adaptive protective immunity. On the other hand, it prevents the development of immunopathological lesions that results from exacerbated immune response [9]. Therefore, both deficiency and overproduction of IL-10 may be responsible for some pathological conditions: low level of IL-10 may result in an increase of inflammatory reactions, in turn, overproduction may result in increased susceptibility to infections. It is believed that approximately 50–70% of the observed variation in IL-10 production is related to genetic factors [10] and that IL-10 levels differ between individuals mainly due to the polymorphisms in the promoter region of the *IL-10* gene [10–14]. The most studied *IL-10* promoter polymorphisms are three biallelic single nucleotide polymorphisms (SNPs) located at −1082 (A/G; rs1800896), −819 (C/T; rs1800871) and −592 (C/A; rs1800972) positions form the transcription start site, which form three different haplotypes GCC, ACC and ATA [11,14–18]. It was determined that −1082GG, −819CC and −592 CC genotypes and GCC haplotype are associated with high IL-10 production (in the case of −1082GG genotype the IL-10 production is twice as high as for AG and AA genotypes). Moreover, it was found that −1082AG, −819CT and −592CA genotypes and ACC haplotype are associated with intermediate, whereas −1082AA, −819TT and −592AA genotypes and ATA haplotype with low IL-10 production [11,14,19]. In regard to −1082, −819 and −592 alleles, association with IL-10 production is ambiguous [13,14,20–22].

It is suggested that genetically determined alterations in interleukin-10 production, are likely to play a role in HCV eradication and affect clinical outcome and the severity of CHC. It was shown that excess IL-10 secretion inhibits protective immunological responses and therefore facilitate viral evasion that may contribute to persistent viral replication [23]. In turn, low IL-10 production is likely to favor HCV spontaneous clearance [24]. Moreover, it is stated that IL-10 exerts anti-inflammatory and antifibrotic action in CHC patients [25,26] and presumably, as a modulator of immune response, may influence the outcome of IFN-based anti-HCV therapy [27].

The aim of our study is to analyze the association between *IL-10* SNPs and severity of liver disease as well as treatment outcome in Polish chronic hepatitis C patients infected with HCV genotype 1. The analysis included *IL-10* promoter SNPs: −1082(A/G) rs1800896, −819(C/T) rs1800871 and −592(C/A) rs1800872 which may determine IL-10 production as well as *IL-10* 3′UTR SNP at position +4529(A/G) rs3024498, which has so far only been analyzed regarding spontaneous HCV clearance [28]. The goal was realized through the analysis of relationship between above-mentioned *IL-10* SNPs and degree of inflammation (G, grading) and stage of fibrosis (S, staging) as well as SVR and relapse after PEG-IFN-α and RBV combined therapy.

2. Material and methods

2.1. Patients

One hundred and ninety-six Polish patients, of Caucasian ethnicity from the region of Wielkopolska, diagnosed with chronic hepatitis C, infected with HCV genotype 1 were included in the study. All patients were treatment-naïve. The exclusion criteria were coexistence of hepatitis B and human immunodeficiency virus infection as well as other chronic liver diseases.

The study included 82 women and 114 men in age 20–64 (median: 39). The liver biopsy, which provides information on both the grade (degree of inflammation that reflects ongoing liver disease injury) and the stage (amount of currently established fibrosis), was performed in 114 patients. The histologic status of liver biopsy specimens was scored using the Scheuer scoring system. Biochemical parameters analyzed at the start of therapy included baseline level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBIL).

All patients were qualified for treatment with standard doses of PEG-IFN-α-2a (PEGASYS®, Roche; 180 or 135 μg per week; n = 78) or PEG-INF-α-2b (PEGINTRON®, Schering-Plough; 1,5 μg/kg of body mass per week; n = 118) combined with weight-based dose of RBV (COPEGUS®, Roche or REBETOL®, Schering-Plough; 1000 mg per day if body weight was <75 kg or 1200 mg per day if body weight ⩾75 kg) for 48 weeks.

Blood samples were obtained before (on the day of treatment initiation), at week 4, 12, 24 and 48 of treatment as well as 24 weeks after the end of treatment. In 62 cases buccal swabs samples were obtained before therapy initiation.

The study was approved by the local ethical committee of the Poznan University of Medical Sciences (no. 650/12). All experiments were carried out in compliance with the relevant laws and guidelines in accordance with the ethical standards of the Declaration of Helsinki.

2.2. DNA extraction

Peripheral blood mononuclear cells (PBMCs) were isolated from 5 ml of venous ethylenediaminetetraacetic acid (EDTA)-blood by Histopaque®-1077 (Sigma-Aldrich, USA) gradient centrifugation (1.077 g/ml), washed twice with phosphate-buffered saline (PBS), and counted. Genomic DNA was extracted from 1×10^6 PBMCs using QIAamp® DNA Mini and Blood Mini Kit (Qiagen, Germany) or from buccal swabs using Invisorb® Spin Tissue Mini Kit (Stratec molecular, Germany), according to the manufacturer's instructions. Quality and quantity of isolated DNA were assessed spectrophotometrically. Next, DNA was diluted to working concentration.

2.3. IL-10 SNPs genotyping

The genotyping of SNPs −1082(A/G) rs1800896 was carried out by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). While −819(C/T) rs1800871, −592(C/A) rs1800872 and +4529(A/G) rs3024498 polymorphisms were determined by high resolution melt (HRM) analysis. To confirm the PCR-RFLP and HRM genotyping results, PCR products from at least 15% of samples for each SNPs were further purified with thermosensitive Exonuclease I and FastAP Alkaline Phosphatase (Fermentas, Thermo Fisher Scientific, USA) and sequenced with BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI Prism 3130XL Analyzer (Applied Biosystems, Foster City, CA, USA) according to manufacturers' protocols. Primer sequences and corresponding PCR product size are shown in Table 1 (Supplementary data).