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Response to Combination Therapy with Interferon Alfa-2a and Ribavirin in Chronic Hepatitis C According to a TNF-α Promoter Polymorphism

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Key Words
Chronic hepatitis C · Interferon alfa-2a · Ribavirin · TNF-α

Abstract
Background: Tumor necrosis factor-α (TNF-α) is involved in the pathogenesis of chronic active hepatitis C. Polymorphisms in the promoter region of the TNF-α gene can alter the TNF-α expression and modify the host immune response. The present study aimed at the correlation of the G308A TNF-α polymorphism with the response to antiviral combination therapy in chronic hepatitis C. ***Patients and Methods:*** 62 patients with HCV and 119 healthy unrelated controls were genotyped for the G308A TNF-α promoter polymorphism. The patients received 3 × 3 million units of interferon alfa-2a and 1,000–1,200 mg ribavirin daily according to their body weight. A response was defined as absence of HCV-RNA and normalization of S-ALT after 6 months of combination therapy. ***Results:*** With respect to the allele and genotype frequency, a significant difference was not observed between controls and patients with chronic hepatitis C. Furthermore, such a difference was also not observed if responders and non-responders to antiviral therapy were compared. ***Conclusions:*** The promoter polymorphism of the TNF-α gene investigated herein is equally distributed in healthy individuals and patients with hepatitis C and does not seem to predict the response to therapy with interferon alfa-2a and ribavirin.

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This work contains parts of the doctoral thesis of C. Simperl.

Introduction

The response to therapy with interferon alfa-2a and ribavirin in chronic active hepatitis C depends on various factors such as age, gender, viral genotype, viral load, duration of infection and presence of liver fibrosis [1]. Cytotoxic effects of multiple cytokines, which confer the inflammatory process in viral hepatitis, might additionally influence the response to antiviral therapy. Moreover, different HLA subtypes [2–4] and the chemokine receptor 5 [5, 6] might be of paramount importance for the pathophysiology of chronic hepatitis C. In addition, cytokines such as interleukin-10 [7] or the tumor necrosis factor-α (TNF-α) [8] seem to influence the course of the disease.

Comparable to various other inflammatory conditions, TNF-α is released in chronic hepatitis C and contributes to the pathogenesis of the liver injury [8, 9]. In particular,

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Table 1. Clinical and laboratory characteristics of the HCV patients and controls

Characteristic	HCV patients (n = 62)	Controls (n = 119)
Female/male	28/34	49/70
Age (mean ± SD)	42 ± 11	39 ± 12
S-ALT, U/l	74 ± 45	normal
Viral load, $\times 10^6$ U/ml	2.46 ± 0.45	–
Genotypes 1 or 4	37	–
Genotypes 2 or 3	25	–

Table 2. Distribution of the –308 TNF-α promoter genotypes in patients and controls

Genotypes	HCV patients	Controls
GG	44 (71%)	86 (73%)
GA	16 (26%)	29 (24%)
AA	2 (3%)	4 (3%)

elevated serum levels of TNF-α mRNA, the respective receptor or increased intrahepatic TNF-α mRNA expression were described in viral hepatitis [8–10]. Two G to A transition polymorphisms, at positions –238 and –308 in the TNF-α promoter region, influence TNF-α expression [11, 12] and were associated with the presence of liver cirrhosis in chronic hepatitis C [13]. In addition, it was suggested that enhanced intrahepatic mRNA levels of TNF-α might reflect resistance to interferon [14], because these levels were significantly higher in non-responders when compared to responders [15]. The latter finding is compatible with intrahepatic TNF-α expression being a host factor that might predict the outcome of antiviral combination therapy with interferon alfa-2a and ribavirin [15].

Hence, the present study evaluated the distribution of the G308A TNF-α promoter polymorphism in controls and patients with hepatitis C and assessed the response to antiviral therapy after stratification for this polymorphism in patients with chronic active hepatitis C.

Patients and Methods

Patients

The study population comprised 62 patients with chronic active hepatitis C and 119 healthy unrelated controls. Overall, 80 patients with chronic hepatitis C had been enrolled in a prospective trial which evaluated the treatment options in patients with concomitant psychiatric disorders, previous drug abuse or current methadone substitution compared with a control group. A detailed description of the study including the number of drop-outs and side effects of the medication has already been reported elsewhere [16].

Inclusion criteria were elevated liver enzymes (alanine aminotransferase, S-ALT >30 U/l) and a positive PCR for HCV-RNA. DNA was available for genotyping from 62 patients. For the correlation between the TNF-α genotype and the virologic response, only patients who completed 6 months of therapy (n = 59) were evaluated in the statistical analysis. The baseline characteristics of the patients and controls are given in table 1. All patients were treated with interferon alfa-2a (Roferon®, Hoffmann-La Roche, Germany) in a dosage of 3 × 3 million units per week combined with ribavirin (Rebetol®, Essex Pharma, München, Germany) in a dosage of 1,000–1,200 mg daily according to their body weight. A response to therapy was defined as normalization of aminotransferases and absence of detectable HCV-RNA after 6 months. The sustained virological response was defined as absence of HCV-RNA 6 months after cessation of antiviral combination therapy. The study was approved by the local Ethics Committee and all patients gave written informed consent.

Determination of the G308A TNF-α Promoter Polymorphism

Genomic DNA was prepared from buffy coat layers employing a commercially available kit (QIAamp DNA Blood Mini Kit, Quiagen, Hilden, Germany). A fragment of the TNF-α promoter gene containing the G308A polymorphism was amplified by polymerase chain reaction (PCR) with a forward primer P1 (5′-AGG CAA TAG GTT TTG AGG GCC AT-3′) and a reverse primer P2 (5′-TCC TCC CTG CTC CGA TTC CG-3′) as described elsewhere [17]. The PCR was performed using HotStar*Taq* DNA Polymerase (Quiagen) in a thermocycler (Biometra, Göttingen, Germany) applying the following conditions: 1 cycle at 95 °C for 15 min, 35 cycles with a denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s and extension at 72 °C for 30 s with a last polymerization step at 72 °C for 10 min. Allele 1 has a guanine (TNF-α 308G), which is substituted by an adenine at position –308 in the polymorphic allele 2.

Restriction analysis was performed with a NcoI digestion which allowed the identification of the separate alleles. The PCR products were analyzed on a 4% NuSieve gel (BioProducts, Rockland, Me., USA).

Statistics

All data are given as mean ± 1 SEM. Statistical analysis was performed using Fisher's exact test and Mann-Whitney U test, respectively. The influence of the G308A TNF-α polymorphism on the response to therapy was evaluated using Fisher's exact test. Results were then confirmed calculating multiway frequency tables employing a log-linear model using BMDP software, version 7.0. p values of <0.05 were considered significant.

Results

Among patients with hepatitis C the allele –308 A was less frequent (20/124 = 16%) than the wild-type allele TNF-α 308G (104/124 = 86%), which was comparable to the distribution in controls (15 vs. 85%, n.s.). The geno-

type distribution in patients and controls was nearly identical (table 2).

59 patients completed 6 months of therapy with interferon alfa-2a and ribavirin. 32 (54%) (15 with HCV genotypes 1/4, 17 with HCV genotypes 2/3) were responders and 27 (46%) (21 with HCV genotypes 1/4, six with HCV genotypes 2/3) were non-responders. Among patients with genotypes GA or AA, the number of responders (n = 9) and non-responders (n = 9) was identical (fig. 1a). Among homozygous carriers of the wild-type allele (GG), 23 patients (56%) were responders and 18 (44%) were non-responders (p = 0.44) after 6 months of therapy (fig. 1a). One patient with GA and 4 patients with GG genotype experienced a relapse 6 months after cessation of antiviral combination therapy. However, a significant difference was also not observed if the sustained virological response was considered (fig. 1b).

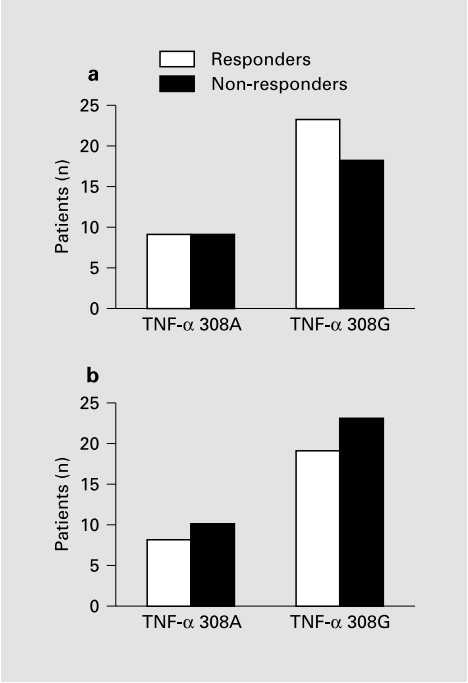

Fig. 1. a Virologic response according to the G308A TNF-α promoter polymorphism after 6 months of therapy. Homozygous (AA) and heterozygous (GA) carriers are classified as TNF-α 308A, homozygous carriers of the wild-type allele as TNF-α 308G. **b** Sustained virologic response according to the G308A TNF-α promoter polymorphism (6 months after cessation of therapy). Homozygous (AA) and heterozygous (GA) carriers are classified as TNF-α 308A, homozygous carriers of the wild type allele as TNF-α 308G.

Discussion

In hepatitis C the host immune response is of pivotal importance for the course of the disease. Thus, it appears possible that polymorphisms of genes which impair the expression of cytokines might be associated with chronic hepatitis C. In particular, the distribution of these polymorphisms in patients with chronic hepatitis C as compared to healthy controls and the response to therapy in carriers vs. non-carriers might be of interest. TNF-α is a pro-inflammatory cytokine which plays a key role in the host immune response. The gene for TNF-α is situated within the MHC class III region on chromosome 6p21.3 [18]. A bi-allelic G (TNF1 allele) to A (TNF2 allele) polymorphism at position 308 results in elevated TNF-α levels [19]. Carriers of this allele are prone to autoimmune disorders like systemic lupus erythematosus, insulin-dependent diabetes mellitus, celiac disease, autoimmune hepatitis, primary sclerosing cholangitis [20–22] and infectious diseases such as leprosy and tuberculosis [23, 24]. In addition, the TNF2 allele was associated with a complicated course following liver transplantation [25, 26]. Lastly, this polymorphism is essential for the immune response in lymphoid malignancies (e.g. non-Hodgkin's lymphoma) and aplastic anemia and influences treatment outcome [27, 28]. The TNF2 allele was also associated with the progression of neurodegenerative diseases [29]. Based on these observations, the G308A TNF-α promoter polymorphism might be of relevance for the susceptibility, the course and the treatment outcome in chronic active hepatitis C [8–10].

However, a correlation between the TNF2 allele and the presence of chronic hepatitis C or the response to antiviral therapy was not observed herein, even if sustained response rates were considered. These findings confirm and extend the results of recently published studies [30, 31]. On the other hand, further genes, such as TGF-β, appear to be associated with resistance to antiviral treatment [32, 33]. Hence, a definite conclusion about the impact of the host immune response for the course of hepatitis C cannot be drawn yet.

Nevertheless, the currently available data argue strongly against a correlation between the G308A TNF-α promoter gene polymorphism and the susceptibility to chronic hepatitis C or the outcome following combination therapy with interferon alfa-2a and ribavirin.

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