Polymorphism of Promotor Region of the Tumor Necrosis Factor-α Gene in Patients with Viral Hepatitis C

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Study of the pathogenesis of viral hepatitis C is of primary importance because of persistence of this virus and high incidence of chronic course of this disease, and as a consequence, development of cirrhotic and neoplastic processes in the liver determining high mortality from this condition. Proinflammatory cytokines, in particular, tumor necrosis factor, play an important role in the development of these pathological processes. The content of tumor necrosis factor in the circulating blood plasma and hepatocytes increases in acute and chronic hepatitis C. It seems that the capacity of cells to produce proinflammatory IL in high or low levels spontaneously or after antigenic stimulation largely determines the outcome of infectious process in contact with the virus.

Key Words: *HCV; necrosis factor-α gene; gene polymorphism; promotor region; individual drug therapy*

Persistence and chronic course of viral hepatitis C are characterized by a wide spectrum of clinical manifestations, eventuating in some patients by the development of decompensated cirrhosis or hepatocellular carcinoma. Cirrhosis of the liver develops in about 20% infected individuals at a certain stage of the disease, while in other patients the disease transforms into asymptomatic phase [12,14]. The dynamics of these processes varies within a wide range and depends on the treatment efficiency and on activity of cell-mediated antiviral immune reactions, compensatory and regeneratory functions of the liver, *etc*. The functions of the immune system are genetically regulated, and it is logical to investigate immunogenetic aspects of the tempo of viral hepatitis progress. These studies were devoted mainly to the analysis of polymorphism of HLA gene alleles in patients with various patterns of viral hepatitis [2,6]. The authors present contradictory information on the distribution of allele variants in patients with chronic hepatitis C, but some associations of DR3 and DR13 with different activity of the infectious process were detected [6]. We previously demonstrated increased incidence of HLA-A10 and HLA-DR5 antigens in Caucasian patients with viral hepatitis in Siberia in comparison with the control [1].

Tumor necrosis factor-α gene (*TNFA*) is located in chromosome 6 between HLA-B and HLA-DR genes, and hence, investigation of its polymorphic regions in parallel with human histocompatibility complex attracts special interest in studies of human diseases. The product of this gene serves as an immunological mediator of inflammatory response to viral exposure and of apoptosis; in addition, it is involved in the development of hepatic fibrogenesis [4,5]. The expression of this gene is controlled at both transcription and posttranscription levels. Two transitions G for A in the gene promotor region (in positions -238 and -308) are essential for the expression and production of TNFα protein. It was shown also that allele 2 in position -308 (A; TNF308.2)

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is associated with increased expression of this gene and increased production of the factor *in vitro* [13].

Increased production of TNFα is a common feature characteristic of autoimmune and infectious diseases. Some studies demonstrated a relationship between promotor variants of *TNFA* gene and clinical outcome of cerebral malaria, biliary cirrhosis, tuberculosis, *etc.* [7,9]. The production of proinflammatory cytokines (including TNFα) increases in response to hepatitis C virus (HCV) infection.

We studied *TNFA* G-308A polymorphism in HCV-infected patients and healthy individuals.

MATERIALS AND METHODS

Thirty-one patients with chronic viral hepatitis C hospitalized at Municipal Infectious Hospital No. 1 in Novosibirsk were examined. The presence of summary anti-HCV served as the selection criterion. A representative age- and sex-matched control group consisted of 52 healthy subjects. Both groups consisted of Caucasians living in Siberia.

Genome DNA was isolated from peripheral blood cells by desalting and proteinase K treatment as described previously [3]. Polymorphic locus G-398A TNFα was typed using *Bsp 191* restrictase (Sibenzim). Amplification of the DNA site containing the above specified polymorphic region was carried out by PCR: 5 min at 94°C, 35 cycles of 30 sec at 95°C, 30 sec at 57°C, 30 sec at 72°C, and final elongation 3 min at 72°C. The reaction was carried out in 20 μl of standard reaction mixture using *Taq*-DNA polymerase (Sibenzim). The primers were synthesized in Vektor Firm. Plasmid *pUC19* cleaved with *MspI* restrictase (Sibenzim) was used as the marker of DNA size. Restriction was carried out for 6 h at 37°C. Restriction products were separated by electrophoresis in 2% agarose gel, stained with ethidium bromide, and visualized in UV light. The length of PCR product was 117 b.p. for variant A, the lengths of two products of *Bsp 191* cleavage in the presence of variant G were 97 and 20 b.p.

The results of genetic studies were processed using odd ratios (OR) with calculation of 95% confidence interval (CI). The distribution of genotypes by the studied polymorphic locuses was verified for correspondence to the Hardy-Weinberg equilibrium (HWE) with the use of precise Fisher's test.

RESULTS

Our data on the distribution of *TNFA* genotypes in the control group show that for this polymorphic locus, in which G/G genotype occupies a dominating position, it is in general similar to the picture in Caucasians in other countries [10]. This characterizes the above-mentioned polymorphism as race-specific.

Distribution of genotypes by the studied genes in both samplings corresponded to that expected according to HWE. Analysis of the data for patients with viral hepatitis C showed for the first time significant differences in the incidence of allele variants of G-308A polymorphism in the promotor region of *TNFA* gene (Table 1). Heterozygotic G/A variant was several times more incident in the patients (35.5%) than in healthy subjects (OR=7.7%, p=0.0015). Rare homozygotic A/A variant was detected in only one individual from the control group (1.9%), but in none of the patients. The incidence of rare allele -308A was significantly higher among HCV-infected patients (OR=3.52; CI 1.12-11.48), which attested to its involvement in the formation of genetic liability to development of infectious processes.

Previous study of the distribution of allele variants of *TNFA* gene carried out by German scientists failed to detect significant differences in the content of G-308A polymorphism genotypes in healthy subjects and patients infected with HCV (but they found allele associations with the disease for transition in locus -238) [7]. No appreciable differences in the incidence of *TNFA* alleles -308 were detected in a group of HCV-infected patients belonging to different races [11]. This confirms that genetic associations with multifactorial diseases depend on ethnic background, because coupling with pathological phenotype was established for different locuses in representatives of different races and peoples.

The studied transition is located in the promotor region of *TNFA* gene and its phenotypical effect con-

TABLE 1. Percentage of Genotypes and Incidence of Alleles G and A by *TNFA* Gene Polymorphism G-398A in Patients with Viral Hepatitis C and Normal Subjects ($M \pm m$)

Group	G/G	G/A	A/A	G	A
Patients with viral hepatitis C	64.5	35.5	0.0	0.82	0.18
Normal subjects	90.4	7.7	1.9	0.94	0.06
OR	0.19	6.6	0.00	0.28	
CI 95%	0.05-0.71	1.66-28.35	0.00-29.83	0.09-0.89	
p	0.0038	0.0015	0.44	0.0138	

sists in alteration of the gene expression and, hence, level of its protein product (TNFα). The role of TNFα in the pathogenesis of HCV is explained by induction of this factor in hepatocytes during infection and increased level of the corresponding protein product in the serum and mononuclears [6-8]. The presence of HCV RNA in hepatocytes and peripheral blood is associated with elevated expression of TNFα [8]. The level of TNFα mRNA in the liver and peripheral blood mononuclears was appreciably lower in patients responding to IFN-α treatment in comparison with patients, whose clinical status did not improve [8]. Previous study found no association between *TNFA* gene polymorphism in HCV patients (belonging to different races) and response to combined or monotherapy with IFN [11]. It can be hypothesized that polymorphism in *TNFA* gene -308 position in Caucasians is pathogenetically significant, and presumably, it is possible to modulate the course of viral hepatitis C by increasing antiviral activity of the immune system via modification of the level of TNFα expression. The data accumulated by the present time indicate that TNFα effects on the course and outcome of HCV infection deserve further investigation, which is now impeded by the absence of appropriate *in vitro* system for HCV.

Hence, the presence of a certain set of allele variants of cytokine genes, for example TNFα gene located in the promotor regions and hence, determining the level of spontaneous and inducible IL production by cells can be essential for the outcome of the host contact with HCV and for the course of infectious process and efficiency of cytokine therapy.

It can be hypothesized that patient genotype in the zone of location of polymorphic regions of cytokine genes and other immune response genes is essential for determining the strategy of antigen-nonspecific immunotherapy and, in the future, individual drug therapy in general.

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