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Association of Immune System Gene Polymorphisms with Quantitative Traits Pathogenetically Important for Chronic Virus Hepatitis

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Abstract—The *IL4* C(–590)T, *IL4RA* Ile50Val, and *TNF* G(–308)A polymorphisms were tested for association with quantitative traits important for chronic virus hepatitis, including the levels of IL4, IL10, IL12, TNF- α , fibronectin, collagenase, the proteinase inhibitor, macroglobulin, and free and protein-bound (PBO) oxypoline. Allele A of the *TNF* G(–308)A polymorphism was associated with a lower TNF- α production by mononuclear cells, a higher production of IL4 and IL12, and a lower PBO level. The genotype CT of the *IL4* C(–590)T polymorphism was associated with a high PBO level.

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INTRODUCTION

To predict the course and outcome of liver diseases, it is necessary to better understand the mechanisms of fibrogenesis and the factors that initiate it. Biochemical reactions leading to fibrogenesis involve many proteins, including proteolytic enzymes (collagenase and elastase); factors controlling their metabolism (interleukins (ILs) 1, 4, 6, 10, 11, and 13; interferons γ and β ; transforming growth factor α ; and tumor necrosis factor α (TNF- α), as well as fibronectin, which reflects the regeneration of liver tissue [1–4]. Fibrogenesis depends on the individual genetic characteristics that affect the functions of all systems both in the norm and upon exposure to various damaging factors.

The genetic basis of predisposition to various infectious diseases has been actively studied since the late 1990s. Many genes have been tested for association with the disease phenotype and qualitative and quantitative traits important for pathogenesis. In particular, cytokine genes have been studied in hepatitis B and C [3] with respect to their severity, progression, response to therapy, and outcome [5–9]. However, very few data are available on the effect of certain gene polymorphisms on the quantitative traits important for the development and progression of chronic virus hepatitis (CVH). Such studies can refine the structure of genetic predisposition to a disease and, in

many cases, explain the association of genetic polymorphisms with the disease and the character of its progression. For instance, *SLC11A1*, *VDR*, *IL1B*, *IL1RA*, and *IL12B* polymorphisms have not been associated with tuberculosis in Tuvinians and Russians, but have been found to affect the size of infiltration foci, destruction of lung tissue, the size of destruction cavities, and cytological and biochemical parameters of the blood. The polymorphisms are consequently considered to be markers that determine the course of tuberculosis and its endophenotypes [10]. *SLC11A1*, *IL1B*, and *IL1RA* polymorphisms have not been associated with tick-borne encephalitis; however, their single nucleotide polymorphisms are associated with the antigenic load of the tick-borne encephalitis virus and the increase in IgM and IgG titers [11]. Such data indicate that the etiological role of the genes examined is uncertain, but the specifics of their function (the amount and qualitative composition of the expression products) may substantially affect the course of various pathologies.

In view of the above, the objective of this work was to test the *IL4* C(–590)T, *IL4RA* Ile50Val, and *TNF* G(–308)A polymorphisms for association with quantitative traits affecting fibrogenesis in the liver.

Table 1. Cytokine production as dependent on the genotype at the polymorphisms under study in CVH patients*

Gene	Genotype	IL4	<i>p</i>	IL10	<i>p</i>	IL12	<i>p</i>	TNF- α	<i>p</i>
<i>IL4RA</i>	II	37.2 \pm 7.3	0.716•	232.6 \pm 39.2	0.323	203.7 \pm 35.1	0.425•	74.9 \pm 19.3	0.301•
	IV	44.5 \pm 8.0		172.2 \pm 23.4		241.6 \pm 33.1		125.7 \pm 39.4	
	VV	16.3 \pm 4.2		122.0		317.5 \pm 46.7		248.2 \pm 155.6	
<i>IL4</i>	CC	35.7 \pm 6.7	0.840•	204.5 \pm 35.6	0.654	244.6 \pm 35.1	0.959•	111.1 \pm 43.2	0.828•
	CT	37.4 \pm 7.9		191.2 \pm 29.1		224.7 \pm 35.7		137.8 \pm 49.2	
	TT	46.2 \pm 18.2		130.2 \pm 9.7		208.0 \pm 72.5		71.3 \pm 23.0	
<i>TNF</i>	GG	33.2 \pm 5.6	0.026•	221.7 \pm 32.1	0.327	274.1 \pm 25.1	0.009•	137.9 \pm 36.5	0.018•
	GA	53.9 \pm 11.1		168.0 \pm 28.4		131.9 \pm 34.3		48.7 \pm 16.1	
	AA	38.3 \pm 12.2		126.2 \pm 24.7		168.2 \pm 125.9		31.0 \pm 1.0	

Note: Here and in Table 3, *p* is the significance level estimated by one-way ANOVA or (•) the Kruskal–Wallis test.

*The results are given as $\bar{X} \pm$ S.E.

EXPERIMENTAL

The sample included 60 CVH patients. The inclusion criteria were type-B and/or type-C CVH serological markers detected in the serum by ELISA (with an ELISA kit) and hepatitis B virus DNA or hepatitis C virus RNA detected by the polymerase chain reaction (PCR).

The ability of peripheral blood mononuclear cells to produce IL4, IL10, IL12, and TNF- α and the serum contents of fibronectin, collagenase, proteinase inhibitor α_1 , macroglobulin α_2 , elastase, and free and protein-bound (PBO) oxyproline were assayed by standard methods [12–15].

The association with the *IL4* C(–590)T, *IL4RA* Ile50Val, and *TNF* G(–308)A polymorphisms was studied by comparing the quantitative traits for carriers of different genotypes. The distribution of quantitative traits was tested for normality by the Kolmogorov–Smirnov test. One-factor ANOVA was used in the case of the normal distribution and the Kruskal–Wallis and Mann–Whitney tests were used in the case of deviations from the normal distribution [16].

DNA of peripheral blood lymphocytes was isolated by the standard nonenzymatic method [17]. Genotyping was performed by restriction fragment length polymorphism (RFLP) analysis of PCR products [18, 19].

RESULTS AND DISCUSSION

The *IL4* C(–590)T and *IL4RA* Ile50Val polymorphisms were not associated with the IL4, IL10, IL12, and TNF- α levels (Table 1). Individuals with different genotypes at the *TNF* G(–308)A polymorphism differed in the distributions of IL4, IL12, and TNF- α levels (Table 1). Given that different alleles of the *TNF* G(–308)A polymorphism determine different production levels of these cytokines, it is possible to expect that their levels linearly change from homozygotes of

one class (GG) through heterozygotes (GA) to homozygotes of the other class (AA). However, the greatest deviations were observed in GA heterozygotes, who displayed the highest IL4 and the lowest IL12 levels (Table 1); only the TNF- α level gradually decreased from homozygotes GG to homozygotes AA (Table 1). Data on the effect of the *TNF* G(–308)A polymorphism on TNF- α production are discrepant. Allele A has been associated with a higher expression of *TNF* and a higher level of TNF- α production in cultured human Raji B cells [20]. On the other hand, this allele has been associated with a lower level of serum TNF- α in patients with chronic obstructive lung disease [21]. It seems that the association of allele A with protein production in vitro and in vivo, especially in different populations, is affected by the linkage of the *TNF* G(–308)A polymorphism with neighbor genome regions. For instance, *TNF* is close to *HLA-DR*, alleles HLADR2 and HLADR3, which are associated with high and low levels of TNF- α , respectively [20].

To verify the association between the quantitative traits and the alleles of the *TNF* G(–308)A polymorphism, we pooled the genotypes containing allele A (GA and AA). Carriers of allele A had a higher serum content of IL4 and a lower content of IL12 and TNF- α as compared with carriers of genotype GG (Table 2). This result demonstrates that the different alleles of this polymorphism affect, to a certain extent, the IL4 and IL12 production. The association of the *TNF* G(–308)A polymorphism with the TNF- α level in the serum is quite conceivable. Rare allele A is a stronger activator of *TNF* transcription and, consequently, is associated with a higher TNF- α production and a more severe course of infectious diseases, such as malaria and leishmaniasis [20]. Allele A is associated with a lower TNF- α level in CVH patients from Tomsk (Tables 1, 3), determining a lower intensity of inflammation and fibrogenesis in the liver. This explains our previous observation that allele A occurs at a higher frequency in CVH with mild fibrosis [22].

Table 2. Association of the *IL4* and *TNF* genotypes with pathogenetically important quantitative traits ($\bar{X} \pm SE$)

Gene	Genotype	IL4	<i>p</i>	IL12	<i>p</i>	TNF- α	<i>p</i>	PBO	<i>p</i>
<i>TNF</i>	GG	33.2 \pm 5.6	0.032	274.1 \pm 25.1	0.003	137.9 \pm 36.6	0.025	10.5 \pm 0.6	0.039
	GA + AA	50.8 \pm 9.2		138.4 \pm 33.9		46.4 \pm 13.9		8.3 \pm 0.8	
<i>IL4</i>	CC + TT	–		–		–		8.5 \pm 0.7	0.008
	CT								

Note: *p* is the significance level estimated by the Mann–Whitney test.

Table 3. Activity of proteolytic enzymes and the contents of fibronectin and the oxyproline fractions in the blood serum of CVH patients as dependent on the genotype at the polymorphisms under study

Gene	Geno- type	Colla- genase	<i>p</i>	Fi- bronec- tin	<i>p</i>	α_2 - macro- globu- lin	<i>p</i>	Elastase	<i>p</i>	Free oxy- proline	<i>p</i>	PBO	<i>p</i>
<i>IL4RA</i>	II	4.46 \pm \pm 0.4	0.393	172.7 \pm \pm 14.2	0.805	2.5 \pm \pm 0.2	0.536	187.1 \pm \pm 15.0	0.207•	1.46 \pm \pm 0.1	0.739	8.4 \pm \pm 0.9	0.759•
	IV	4.28 \pm \pm 0.3		165.2 \pm \pm 9.5		2.7 \pm \pm 0.2		157.1 \pm \pm 11.1		1.49 \pm \pm 0.1		10.7 \pm \pm 0.7	
	VV	3.48 \pm \pm 0.4		181.0 \pm \pm 12.8		5.2 \pm \pm 1.2		194.8 \pm \pm 34.4		1.62 \pm \pm 0.2		8.6 \pm \pm 1.6	
<i>IL4</i>	CC	4.25 \pm \pm 0.3	0.235	181.9 \pm \pm 6.6	0.296	3.04 \pm \pm 0.5	0.241•	167.3 \pm \pm 11.9	0.829•	1.52 \pm \pm 0.1	0.860	8.4 \pm \pm 0.8	0.071•
	CT	4.18 \pm \pm 0.3		166.9 \pm \pm 13.4		2.5 \pm \pm 0.2		184.7 \pm \pm 17.0		1.54 \pm \pm 0.1		11.4 \pm \pm 0.8	
	TT	5.89 \pm \pm 1.3		110.0		4.1 \pm \pm 1.1		200.2 \pm \pm 42.3		1.68 \pm \pm 0.4		8.9 \pm \pm 1.5	
<i>TNF</i>	GG	4.41 \pm \pm 0.3	0.647	165.5 \pm \pm 9.3	0.961	3.0 \pm \pm 0.3	0.509•	176.1 \pm \pm 11.6	0.583•	1.47 \pm \pm 0.1	0.094	10.5 \pm \pm 0.6	0.044•
	GA	3.93 \pm \pm 0.4		169.3 \pm \pm 12.3		2.5 \pm \pm 0.3		175.5 \pm \pm 18.2		1.72 \pm \pm 0.1		8.2 \pm \pm 0.8	
	AA	4.16 \pm \pm 1.3		168.3 \pm \pm 32.7		2.4 \pm \pm 0.8		145.1 \pm \pm 50.3		0.80		10.8	

Allele A proved to be associated, not only with higher TNF- α production, but also with a higher content of IL4, which affects antibody production, limits the distribution and intensity of inflammation, and serves as a key signal determining the Th-2 immune response in CVH [5]. The opposite effects of allele A on the IL4 and TNF- α production testifies again that mutual inhibition of pro- and anti-inflammatory cytokines is genetically determined. Allele A exerts a protective effect, determining a milder course of CVH.

The *IL4* C(-590)T and *IL4RA* Ile50Val polymorphisms were tested for associations with the activity of proteolytic enzymes and the serum contents of fibronectin and the fractions of oxyproline (collagen

metabolite). The *TNF* G(-308)A and *IL4* C(-590)T polymorphisms proved to be associated with the PBO content (Tables 2, 3). When the *TNF* genotypes GA and AA were pooled, carriers of allele A displayed a lower PBO level as compared with carriers of genotype GG (Tables 2, 3).

Serum PBO is directly associated with the intensity of collagen synthesis in the liver. Hence, allele A, associated with a lower PBO content, determines a lower intensity of collagen synthesis. This finding supports the protective role of allele A with respect to fibrosis in CVH.

Heterozygous genotype CT of the *IL4* C(-590)T polymorphism has been associated with liver cirrhosis in CVH [22] and, on the other hand, tends to deter-

mine a higher PBO level than that in carriers of homozygous genotypes CC and TT (Table 3). To verify the significance of genotype CT for the PBO level, we pooled the homozygous genotypes CC and TT (Table 2).

Heterozygous genotype CT of the *IL4* C(-590)T polymorphism was associated with a higher PBO level (Table 2). This suggests intense collagen synthesis for CVH patients carrying genotype CT; i.e., this genotype is an unfavorable marker of CVH progression. Our findings explain the association of genotype CT with cirrhosis in CVH patients [22]. It is possible that virus hepatitis patients with genotype CT have a tendency for disease progression and cirrhosis development.

To summarize, allele A of the *TNF* G(-308)F polymorphism was associated with a higher production of *TNF- α* , a lower production of *IL4* and *IL12*, an a low PBO level, suggesting a low rate of collagen synthesis in the liver. Allele A seems to determine a milder course of CVH. Genotype CT of the *IL4* C(-590)T polymorphism was associated with a high PBO content, suggesting active synthesis of collagen in the liver. This explains the association of genotype CT with cirrhosis as the ultimate stage of liver fibrosis [22] and identifies this genotype as a marker of a more severe CVH course.

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