Polymorphism of Promotor Sites of Interleukins-4 and -10 and Tumor Necrosis Factor-α Genes in HIV-Infected Patients

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The distribution of allele variants of promotor sites of interleukins 4 (C-590T) and 10 (C-597A) and tumor necrosis factor-α (G-308A) genes was studied in HIV-infected patients and normal subjects of the Europeoid population in Russia. Some deviations in the distribution of genotypes of the studied polymorphism were revealed in HIV-infected patients compared to the control. The distribution of genotypes in these groups is different for men and women, which is significant for inheritance of allele variants of the cytokine gene.

Key Words: *interleukins; tumor necrosis factor-α; gene polymorphism; HIV infection; Russian population*

The content of various cytokines and the function of the cytokine system are disturbed in HIV-infected patients [13]. Increased production of interleukins 4 and 10 in parallel with decreased synthesis of interleukin-2 suggest that cytokine imbalance (transition from Th1-dominant to Th2-dominant cytokine profile) plays an important role in AIDS progression [11]. Tumor necrosis factor-α plays a special role in the pathogenesis of AIDS: it stimulates replication of HIV and promotes horizontal transmission of the infection [3]. Effects of interleukins 4 and 10 on HIV replication *in vitro* were previously reported [11].

Allele gene polymorphism of many known cytokines, effects of allele variants of genes on characteristics and function of the corresponding proteins were previously described [5]. Some allele variants of cytokine genes are associated with high susceptibility to autoimmune, allergic, and infectious diseases, characteristic features and severity of their course, and increased or decreased content of the cytokines [4,7]. The presence of variant 308A in the tumor necrosis factor-α (*TNF*-α) gene promotor is associated with its increased production by immune cells [8], while 590T allele in interleukin-4 (*IL-4*) gene correlates with increased production of this cytokine and rapid progress of HIV infection [13].

We investigated the distribution of *IL-4*, *IL-10*, and *TNF*-α allele variants in HIV-infected patients and the distribution of genotypes in men and women.

MATERIALS AND METHODS

A total of 120 HIV-infected patients (35 women and 85 men) aged 16-51 years and 52 normal subjects (25 women and 27 men) were examined. Both groups consisted of representatives of phenotypically Europeoid population of Russia.

High-molecular-weight genome DNA was isolated from peripheral blood cells [6]. DNA site containing the specified polymorphism was amplified by PCR using appropriate primers and parameters of thermal cycles [5,12,14]. The reaction was carried out in 20 μl standard reaction mixture containing *Taq*-DNA polymerase (Sibenzim). Plasmid *pUC19* cleaved by *MspI* restrictase (Sibenzim) was used as DNA size marker. *IL-1* C590T polymorphism was typed using *Bsm FI* restrictase (New England Biolabs Inc.), *IL-10* A-597C polymorphism using *Rsa I* (Sibenzum), and *TNF*-α A-308G polymorphism using *Bsp 19I* (Siben-

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zim). Restriction of *IL-4* amplification product was carried out for 6 h at 65°C, restriction of *IL-10* and *TNF*-α gene products for 10-12 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 *IL-4* PCR product was 252 b.p. for variant T, the length of two products of *Bsm FI* cleavage in the presence of variant C was 192 and 60 b.p., the length of *Rsa I* cleavage products in the presence of variant A was 240, 232, 66, and 42 b.p. and in the presence of variant C 306, 232, and 43 b.p. The length of *TNF*-a gene PCR product was 117 b.p. for variant A, the length of two products of *Bsp 19I* cleavage in the presence of variant G was 97 and 20 b.p.

The results were statistically processed using Woolf relative risk (RR) parameters and Gubler prognostic coefficient [1]; the significance of differences was evaluated using Fisher's precise method [3].

RESULTS

Homozygous A/A variant of *IL-10* (RR=2.36) was detected in HIV-infected patients. This variant was not detected in healthy controls; homozygous A/A variant of *TNF*-α (RR=-12.25) was not detected in the patients and was detected in 4% healthy women. *IL-4* genotype T/T and *TNF*-α A/G variant were 4-fold more incident in HIV-infected patients (RR=4.67) at the expense of lower incidence of both homozygous variants (Table 1).

Homozygous C/C variant of *IL-4* C590T polymorphism predominated in donors (RR=2.36; p= 0.0155); this reflected the general distribution of genotypes in other Europeoid populations, in contrast to Mongoloid populations, in which C/T variant predominated [5,9]. *IL-10* C-597A polymorphic site was also characterized by the typical Europeoid predominance of genotype C/C (RR=7.37; p=0.0001), whose incidence in mongoloids was low [12]. The distribution of *TNF*-α G-308A polymorphism genotypes reflects regularities detected for both Europeoid and Mongoloid populations [7,16], among which G/G genotype predominates (RR=112.8; p=0.0001).

The distribution of the studied cytokine genes in HIV-infected patients and donors was different in men and women. *IL-4* C/C genotype predominated in healthy women (RR=2.67; p=0.054), while in men this difference less pronounced (RR=2.12) because of the absence of T/T genotype. By contrast, the incidence of *IL-10* C/C genotype was more than 4-fold higher in men (RR=19.36; p=0.0001), while in women the difference was less pronounced (RR=3.16; p=0.033). The predominance of *TNF*-α G/G genotype in men (RR=156.25; p=0.0001) in comparison with women (RR=84.33; p=0.0001) was more marked due to the absence of the homozygous A/A variant in men.

In contrast to healthy men, HIV-infected men had *IL-4* T/T genotype, its incidence being higher than in women. However the ratio of two other *IL-4* genotypes in the subgroups of HIV-infected patients was comparable to that in donors. The predominance of *IL-10* C/C and *TNF*-α G/G gene variants, characteristic of healthy men, was far less pronounced in HIV-infected men (RR=4.37 for *IL-10* and RR=7.27 for *TNF*-α). The ratio of gene variants of all three cytokines was stable in both healthy and HIV-infected women, except the rare homozygous variants. The incidence of *IL-10* heterozygous A/C genotype was lowered at the expense of a higher number of women with the rare A/A genotype. None of HIV-infected women had *TNF*-α A/A genotype.

Analysis of the incidence of allele gene variant combinations of the studied cytokines separately in men and women showed that the prognostic coeffi-

TABLE 1. Distribution (%) of Cytokine Genotypes in HIV-Infected Patients and Donors of Different Sex

Genotype		HIV-infected patients			Donors		
		men	women	total	men	women	total
IL-4	C/C	52.9	60.0	54.2	59.3	60.0	59.6
	C/T	40.0*	34.3*	39.2*	40.7	36.0*	38.5*
	T/T	7.1*	5.7*	6.6*	0*	4.0*	1.9*
IL-10	C/C	67.1	62.9	65.7	81.5	64.0	73.1
	A/C	31.7*	28.5*	30.8*	18.5*	36.0*	26.9*
	A/A	1.2*	8.6*	3.3*	0*	0*	0*
TNF-a	G/G	72.9	80.0	75.8+	92.6°	88.0	90.4
	A/G	27.1*	20.0*	24.2*+	7.4*°	8.0*	7.7*
	A/A	0*	0*	0*	0*	4.0*	1.9*

Note. $p \leq 0.05$ compared to: *predominant genotype, +control, °HIV-infected men.

cients were higher, if they were calculated separately for men and women; this made the prognosis more individual and reliable.

Total prognostic coefficient of predisposition to HIV infection by analysis of *IL-4*, *IL-10*, and *TNF*-α polymorphisms with consideration for sex can be 14.1 for men, which corresponds to 96% probability of the prediction realization, and 12.5 (92%) for women. Analysis of a greater number of polymorphisms can increase the reliability of the prognosis to 99%.

Hence, significant differences in the distribution of combinations of allele gene variants in the key cytokines in HIV-infected patients are associated with patient's sex and disease patterns. These data indicate that in case of an accidental contact with the virus, the process of infection depends on immunogenetic factors, which determine in many cases natural resistance to virus penetration into immune system or create conditions for long persistence of the virus in lymphoid cells. Presumably this is explained by effect of the primary structure of the cytokine gene polymorphic promotor sites on the level of production of the regulatory factors by immune cells.

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