Production of TNF-α and IL-1β by Peripheral Blood Mononuclear Cells in Carriers of Different Allele Variants of the Gene

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Associations of cytokine production by mononuclear cells and the TNF-α genetic polymorphism in positions -238, -308, -376, -857, -1031, and of IL-1β in positions -31 and +3954 were studied. The data on distribution of allele and genotype incidence and on the level of spontaneous and mitogen-induced production of these cytokines by donor mononuclear cells demonstrated a statistically significant association of TNF-α production by mononuclear cells with polymorphic variants of the gene promoter regions -238>A (rs361525) and -857C>T (rs1799724). Carriers of -238GG/-308GG/-857CC/-1031NC genotype were characterized by low production of TNF-α, while carriers of -31TT/+3954CT genotype were characterized by low IL-1β stimulation index in response to mitogen in comparison with carriers of other genotype combinations.

Key Words: *cytokines; mononuclear cells; promoter; allele polymorphism*

Changes in the regulatory sequences in the structure of promoter and other regulatory sites of genes, leading to changes in their expression are possible mechanisms of the genetic polymorphism modulation of the gene function. As for the immunoregulatory cytokine genes, polymorphism is essential for the gene expression and the intensity of immune reactions realized through the mediator. Hence, polymorphic genetic sites can be regarded as markers of liability/resistance to diseases, the pathogenesis of which is regulated by the cytokine network [2,3].

High production of proinflammatory cytokines IL-1β and TNF-α is observed in many systemic inflammatory diseases. Some of TNF-α gene polymorphisms are informative as genetic markers of liability to some cancers and autoimmune diseases [1,6]. On the other hand, the mechanisms and effects of certain allele variants of the genes on the levels of these cytokines production by immunocompetent cells are little studied. In addition, the informative value of the same genetic markers is often different for different ethnic groups, and hence, identification of the markers should be carried out for each population.

We studied the relationship of allele variants of IL-1β and TNF-α genes promoter regions and the level of their proteins production by the peripheral blood mononuclears in donors, residents of Novosibirsk.

MATERIALS AND METHODS

The peripheral venous blood specimens were collected from 220 volunteers selected at random at blood transfusion centers in Novosibirsk. Polymorphic sites in gene promoters were selected for molecular genetic study (Table 1) and +3954C>T polymorphism was included located in IL-1β gene exon 5 essential for

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TABLE 1. Primer Sequences, Restriction Enzymes, and Fragment Sizes after PCR and Restriction

Locus	Primer sequence	PCR product, nucleotide pairs	Endonuclease	Restriction products, nucleotide pairs
-31T>C	F: 5'-agaagcttccaccaatactc-3'	239	AluI	T: 136,97,6
(rs1143627)	R: 5'-agcacctagttgtaaggaag-3'			C: 233,6
+3954C>T	F: 5'-ctcaggtgtcctcgaagaaatcaaa	194	TaqI	C: 95,85,14
(rs1143634)	R: 5'-gctttttgctgtgagtcccg			T: 180,14
-238G>A	F: 5'-cccagaagaccccctcggaacc-3'	126	MspI	G: 104, 22
(rs361525)	R: 5'-accttctgtctcggtttcttctccatcgc-3'			A: 126
-308G>A	F: 5'-aggcaataggttttgagggccat-3'	117	Bsp19I	G: 97, 20
(rs1800629)	R: 5'-acactccccatcctcccggc-3'			A: 117
-376 G>A	F: 5'-ccccgttttctctccctcaa-3'	106	Sse9I	G: 106
(rs1800750)	R: 5'-tgtggtctgtttccttctaa-3'			A: 88, 18
-857C>T	F: 5'-aagtcgagtatggggacccccccgttaa-3'	133	Hind II	C: 108, 25
(rs1799724)	R: 5'-ccccagtgtgtggccatatcttctt-3'			T: 133
-1031T>C	F: 5'-aaggctctgaaagccagctg-3'		444	BstV9I
(rs1799964)	R: 5'-cttccatagccctggacattct-3'			C: 300, 144

protein production in monocyte culture [4]. All polymorphisms have been described and their identificators are presented in dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/).

Mononuclears were isolated from the peripheral blood by density gradient centrifugation. The cells in a concentration of 10^6/ml were cultured in RPMI 1640 with 10% FCS with or without mitogens LPS or concanavalin A (ConA) in a final concentration of 10 μg/ml. After culturing the conditioned media were corrected and stored at -20°C before analysis. The cytokines were measured by electrochemiluminescent immunoanalysis on an Origen Analyzer (Igen Inc.) [5]. The production stimulation indexes were calculated as a the proportion of cytokine concentration in conditioned media after culturing of mitogen-stimulated cells to its concentration in conditioned media after culturing without stimulation.

The genome DNA was isolated using Proba-NK kit (DNA Technology). The target gene fragments were amplified by PCR. The reaction mixture (20 μl) contained 0.25 ng DNA, 0.5 μM each primer, 0.25 mM each dNTP, 1 U Taq-polymerase (Sibenzyme) in standard buffer offered by enzyme manufacturer. Polymorphic variants were detected by restriction analysis of PCR products. The DNA fragments for analysis were hydrolyzed by specific restriction endonucleases and visualized after electrophoretic separation in agarose gels stained with ethidium bromide.

The genotype frequency correspondence to Hardy–Weinberg's equilibrium was verified by χ^2 test. The relationship between genotype and production level was evaluated by nonparametric Kruskal–Wallis analysis of dispersions (H). The differences in the level of TNF-α production in carriers of different genotypes were evaluated by Mann–Whitney U test (Z).

RESULTS

The distributions of allele and genotype frequencies (Table 2) were compared by the parameters in other populations using the NCBI database on the studied polymorphisms for the reference populations (in ss66857510): CEU-GENO-PANEL for Caucasians, AAM-GENO-PANEL for African Americans, and CHBGENO-PANEL for the Asian race. The resultant allele incidence and genotype distribution for TNF-α promoter in polymorphic loci -238 and -857 were characteristic of the Asian populations, the incidence for locus -1031 was more characteristic of the Europeans, while the values for locus -308 were different from all Asian and European samples. Base replacement in the promoter position -376 in the sample we studied was extremely rare, which suggested excluding this polymorphism from further analysis. The detected incidence of IL-1β gene promoter alleles for both loci did not differ from those in the European populations.

A significant relationship between TNF-α production and polymorphic variants of the gene promoter region in loci -238G>A and -857C>T was detected. Carriers of -238GG genotype were characterized by

TABLE 2. Incidence of Gene IL-1β and TNF-α Genotypes and Polymorphism Alleles in Residents of Novosibirsk

Polymorphism	*n*	Genotype incidence, % (number)			Allele incidence, %	
IL-1β (-31T>C)	195	TT	TC	CC	T	C
		39 (76)	51.3 (100)	9.7 (19)	64.6	35.4
IL-1β (+3954C>T)	195	CC	CT	TT	C	T
		57.4 (112)	36.4 (71)	6.2 (12)	75.6	24.4
TNF-α (-238G>A)	154	GG	GA	AA	G	A
		92.2 (142)	7.8 (12)	0 (0)	96.1	3.9
TNF-α (-308G>A)	220	GG	GA	AA	G	A
		78.6 (173)	20.9 (46)	0.5 (1)	89.1	10.9
TNF-α (-376 G>A)	163	GG	GA	AA	G	A
		99.4 (162)	0.6 (1)	0 (0)	99.7	0.3
TNF-α (-857C>T)	160	CC	CT	TT	C	T
		76.2 (122)	21.3 (34)	2.5 (4)	86.9	13.1
TNF-α (-1031T>C)	162	TT	TC	CC	T	C
		61.7 (100)	36.4 (59)	1.9 (3)	79.9	20.1

low spontaneous production of TNF-α in mononuclear cultures in comparison with -238GA heterozygotes (H=3.8; Z=-1.92; p=0.05), while ConA stimulation of cells markedly stimulated the cytokine production (H=3.95; Z=-1.99; p=0.046; Table 3). Carriers of -857CC genotype were characterized by lower production of TNF-α in mononuclear cultures after cell treatment with ConA (Z=-1.98; p=0.047) in comparison with carriers of the alternative allele.

The absence of statistically significant association of cytokine production by mononuclears *in vitro* with TNF-α -308G>A and -1031T>C and IL-1β -31T>C and +3954C>T polymorphisms does not indicate that they are completely inessential for gene expression. The contribution of these polymorphisms to expression regulation cannot directly influence the final level of protein produced and be realized by regulation of, for example, transcription rate or stability of matrix RNA and be specific for various cells and tissues. We suggested that the effect of genetic polymorphic sites on the gene expression could be realized through their combinations. The results of analysis of associations of genotype combinations with IL-1β (Table 3) and TNF-α (Fig. 1) production levels in mononuclear conditioned media supported this hypothesis. Analysis of IL-1β gene promoter polymorphisms detected 8 genotype combinations of 9 possible ones and demonstrated a statistically significant difference of this cytokine production stimulation indexes in carriers of two variants of combinations. Carriers of heterozygotic variants in both studied loci (-31TC/+3954CT) exhibited a more intense response to ConA stimulation (higher stimulation index) than carriers of the -31TT/+3954CT combination (Z=-2.02, p=0.043; Table 3).

Based on the results of TNF-α gene promoter genotyping, 10 variants of genotype combinations were singled out. Of these, 6 variants were selected, for which the number of individuals with these combinations allowed a statistical processing. The results demonstrated the -238GG/-308GG/-857CC/-1031NC genotype (Fig. 1, *2*) characterized by the lowest produc-

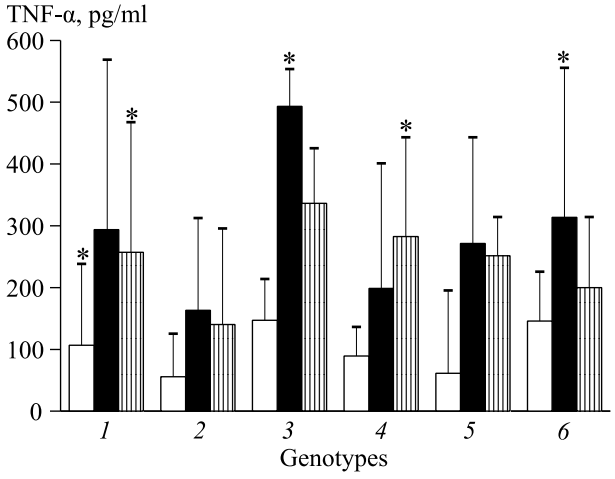

Fig. 1. Level of TNF-α in conditioned media of primary cultures of mononuclears from carriers of different genotype combinations. The data are presented as the median and quartiles. Light bars: cultures without mitogens; dark bars: ConA stimulation; vertically hatched bars: LPS stimulation. Genotype combinations for loci -238/-308/-857/-1031, respectively, are denoted as: *1*) GG/GG/CC/TT; *2*) GG/GG/CC/NC; *3*) GG/GG/NT/NC; *4*) GG/NA/CC/TT; *5*) GG/NA/CC/NC; *6*) GG/GG/NT/TT. *$p \leq 0.05$ in comparison with *2*.

TABLE 3. Indexes of TNF-α and IL-1β Production Stimulation by Mitogen (ConA) in Mononuclear Conditioned Media

Polymorphism or combination	*n*	Genotype	Median	Lower quartile	Upper quartile
TNF-α -238G>A	148	GG	3.05	1.91	5.52
		GA	2.02	1.74	2.98
TNF-α -857C>T	148	CC	2.86	1.75	4.66
		NT (CT+TT)	3.31	1.99	6.74
IL-1β -31T>C/+3954C>T	67	TT/CT	1.18	0.69	3.40
		TC/CT	1.93	1.40	4.80

Note. All values differ significantly ($p \leq 0.05$).

tion of TNF-α by intact (Z=-2.39; p=0.017) and ConA (Z=-2.21; p=0.027) and LPS stimulated (Z=-2.69; p=0.007) mononuclears in culture in comparison with the group of carriers of the rest genotypes. In addition, carriers of genotype *2* exhibited a significantly lower level of TNF-α production by ConA-stimulated mononuclears than individuals with genotypes *3* and *6* (Fig. 1) carrying minor -857T allele. This was in line with the data on polymorphism -857C>T (Table 3). In addition, carriers of genotype *2* had a significantly lower level of TNF-α in conditioned media after LPS stimulation than carriers of genotypes *1* and *4*. This was presumably due to contribution of polymorphism -1031T>C for which no relationship with the level of TNF-α production was revealed in a special analysis; it was presented in combinations *1* and *4* by only wild type alleles (-1031TT).

Hence, evaluation of spontaneous and stimulated production of IL-1β and TNF-α cytokines in cell culture showed allele variants of these genes promoter regions, differently responding to external induction of the expression. This seems to be the most important characteristic for inducible immunoregulatory cytokines.

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