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IMMUNOGENETIC MARKERS OF THE SIBERIA SOUTHERN REGIONS POPULATION UNDER THE EXPOSURE OF TECHNOGENOUS FACTORS

ABSTRACT

Peculiarities of the population health, characteristic for the newly emerging endemic provinces in Russia, are formed by the anthropogenic pollution conditions of the environment and determine the need to identify the indicator of immunological and genetic indicators that reflect the population health of industrial regions.

The aim of the work is to analyze the population immune and genetic indicators in the conditions of the anthropogenic impact of the urban environment on the example of the Southern Siberia region.

Materials and methods. Laboratory immunological and genetic examination of the adult population living in a combined influence of a chemical factors number of habitat zone is carried out. The content of specific antibodies to benzo(a)pyrene, aluminium, formaldehyde was detected by allergosorbent testing with an enzyme label. Cell populations were determined by CD markers on a flow cytometer. Biochemical markers were studied by enzyme immunoassay. Genetic features were detected by real-time polymerase chain reaction and allele discrimination, based on the diagnosis of single-nucleotide polymorphisms.

Results. There was a significant decrease in the number of $CD3^+CD25^+$- lymphocytes relative to the reference interval, and there were lower concentrations of $CD16^+CD56^+$ -, $CD3^+$- and $CD4^+$-cells relative to the comparison group. The decrease in serum IgG and IgM levels was combined with an increase in the level of IgG to aluminium and benzo(a)pyrene, IgE to formaldehyde relative to the values in the comparison group. There was a significant (p=0.02) increase in the frequency of occurrence of the minor allele of the enzyme eNOS rs1799983 gene associated with a decrease in serum levels of nitric oxide, which indicates the formation of additional risk factors under technogenic exposure.

Findings. The established changes in immune reactivity and genetic polymorphism indicate their population peculiarities of the technogenic chemical province of the Southern Siberia region, which can be used as formation of pathological health disorders markers associated with oxygen-associated processes in the vascular endothelium, the implementation of which can contribute to the identified imbalance of cellular and humoral immunity ($CD3^+CD25^+$- lymphocyte deficiency and IgG hyperproduction to aluminum and benzo(a)pyrene, IgE to formaldehyde).

Keywords: immune regulation, genetic polymorphism, *eNOS* gene rs1799983, technogenous factors.

Newly emerging Russian endemic provinces are evolving under the conditions of technogenic environmental pollution and immunological health of the population who live there, especially taking into account individual genetic variability, is subject to study due to necessity to solve treatment and preventive tasks under technogenic transformation of the external environment in the areas under active industrial influence [5, 6, 8, 9].

Chemical environmental pollution might cause the toxic effect on the functions of immune cells, as well as have both immunoactivating and inhibitory effects, leading to the development of immune-mediated diseases which are either allergic or autoimmune ones [1, 10, 14, 15]. It should be noted that the nature and specificity of the resulting changes are usually associated with the exposure features, exposure duration, exposure sources and properties of the factor itself or their combination. Therefore, it is particularly important to study the immunogenetic features of the population, with regard to the combined multi-factor environment, and to determine the indicators of pathogenetic trends related to health disorders [2, 3, 12].

The research **aims** at analyzing the immune and genetic indicators of the population under technogenic impact exerted by the urban environment and exemplified by Southern Siberia region.

Materials and methods. The population living in the industrial center located in the Irkutsk region was examined. The observation group included 50 people living in the zone of technogenic pollution factors (mean age amounts to 34.06±0.84 years). The comparison group consisted of 31 people living on a relatively clean area outside the zone under industrial impacts, remote from the region of technogenic chemical province (mean age is 41.29±2.22 years).

Immunoglobulin concentration (IgG, IgM, IgA) in serum was estimated using radial immunodiffusion (Mancini method). Specific response to the factors related to the chemical burdens was determined with reverse enzyme allergosorbent test according to the level of specific IgG antibodies to aluminum and benzo(a)pyrene, specific IgE antibodies to formaldehyde. The levels of nitrogen oxide and superoxide dismutase in serum were measured with enzyme immunoassay using commercial test systems ("BenderMedSystems", Austria, "RnD Systems", USA). The ratio of lymphocyte populations was determined as per membrane CD markers using panels of monoclonal antibodies to CD receptors ("Becton Dickinson", USA) on FACSCalibur flow cytometer ("Becton Dickinson", USA), taking into account at least 10,000 events.

The data were processed using program Statistica 6.0 (Statsoft, USA), the results were presented as an arithmetic mean and standard error of mean (M±m). Statistical significance was assessed at $p<0.05$ using Student's t-test.

The biological material from the mucous membrane of the oropharynx was obtained for genetic analysis. DNA was extracted by the sorbent method via cells destruction. Genotyping of polymorphisms was carried out using sets of "SNP-screen" ("Synthol", Russia). Genotypes were determined via polymerase chain reaction on CFX96 thermal cycler ("Bio-Rad", USA), using the real-time mode and the allele discrimination method to separate groups as per genotypes. Genotyping data were processed using "Gene Expert" program, genotype frequencies were calculated as per Hardy-Weinberg equilibrium based on the diagnosis of single-nucleotide polymorphisms (SNP). The statistical intergroup significance in the genotype and allele frequency distribution of studied traits was determined by χ^2 criterion, using codominant and multiplicative inheritance models. The data in terms of allele frequencies were analyzed with the calculation of odds ratio (OR, 95%CI).

Results and discussion. The population research showed functional changes in immune indicators in the observation group (table 1). Thus, there was a significant decrease in the number of $CD3^+CD25^+$-lymphocytes relative to the reference interval in 75.9% of the examined ($p<0.05$). As for the comparison group, a lower concentration of $CD16^+CD56^+$-lymphocytes on the average of 1.8-2.3 times, $CD3^+$- and $CD4^+$-lymphocytes at 1.2 times according to the absolute level of marker expression was revealed ($p<0.05$).

At the same time, we may observe a significant change in humoral immunity. The levels of IgG and IgM were reduced by 1.2 and 1.5 times, respectively relative to the values in the comparison group ($p<0.05$).

In the meantime, an increase in the level of specific antibodies was observed when compared with the reference range for IgG marker to aluminum in 64.6% of the examined ($p<0.05$). Besides, the level of IgG antibodies to aluminum increased 2.8 times, IgG to benz(a)pyrene 5.1 times, IgE to formaldehyde 3.0 times compared to the comparison group ($p<0.05$).

Thus, we revealed the changes in the immune reactivity indicators in the examined population under technogenic influ-

Table 1

Indicators of immune regulation in the population under technogenic exposure

Index	Reference interval	Observation group	Comparison group
CD16+CD56+-lymphocytes. 109/dm3	0.09-0.59	0.148±0.033*	0.348±0.098
CD16+CD56+-lymphocytes. %	5-27	7.675±1.469*	13.737±2.644
CD19+-lymphocytes. 109/dm3	0.09-0.66	0.203±0.045	0.269±0.054
CD19+-lymphocytes. %	6-25	9.85±1.102	10.842±1.492
CD3+-lymphocytes. 109/dm3	0.69-2.54	1.426±0.145*	1.762±0.235
CD3+-lymphocytes. %	55-84	76.325±2.284	71.789±2.543
CD3+CD4+-lymphocytes. 109/dm3	0.41-1.59	0.858±0.086*	1.036±0.133
CD3+CD4+-lymphocytes. %	31-60	45.175±2.411	42.684±2.945
CD3+CD8+-lymphocytes. 109/dm3	0.19-1.14	0.535±0.063	0.639±0.116
CD3+CD8+-lymphocytes. %	13-41	28.35±2.341	25.579±2.938
CD3+CD25+-lymphocytes. 109/dm3	0.19-0.56	0.173±0.045	0.129±0.043
CD3+CD25+-lymphocytes. %	13-24	9.448±2.479**	5.158±1.457
IgG. g/cm3	10-18	11.994±0.583*	15.825±1.035
IgM. g/cm3	1.1-2.5	1.558±0.101*	2.318±0.431
IgA. g/cm3	1.1-3.0	2.21±0.15	2.392±0.303
IgE to formaldehyde. IU/cm3	0-1.5	0.305±0.099*	0.103±0.061
IgG to aluminum. cu	0-0.1	0.216±0.053*/**	0.078±0.03
IgG to benzo(a)pyrene. cu	0-0.3	0.248±0.08*	0.049±0.032

Note: * - the difference is significant relative to the comparison group; ** - the difference is significant relative to the reference interval ($p<0.05$).

ence, which are associated with a quantitative imbalance in the main populations of immunocompetent cells, a decrease in the functional activity of humoral immunity with hypersensitivity development to the chemical influence factors regarding the level of specific antibodies to aluminum, formaldehyde, benz(a)pyrene.

The development of predisposition to allergic conditions in the population living on the territories where active technogenic development takes place is determined by the excessive allergenic load of multi-factor industrial pollution. As a result, there is an excessive activation of immune cells, which often leads to the strengthening of oxidative reactions and the development of oxidative stress and pathophysiological states [13, 16].

When performing genetic study of the population living on the observation area, we determined the features of individual genetic variability in the genes of inflammation and antioxidant protection of endothelial nitrogen oxide synthase eNOS (rs1799983) and superoxide dismutase SOD2 (rs2758330) (table 2). The ratio analysis of the genotype and allele frequencies in the examined groups revealed a significant increase in the prevalence of mutant allele T of the eNOS gene in the observation group at the level of 19.7% and at 6.5% in the comparison group ($p<0.05$). At the same time, there were no significant differences in the frequency of occurrence of minor genotypes and alleles in the SOD2 gene. It might be assumed that the presence of the mutant allele T of the eNOS gene acts as an additional risk factor in the population with a high level of allergization under technogenic influence when developing inflammatory processes and oxidative stress (OR=3.566, 95%CI=1.18-11.375), since it is due to a decrease in the basal production of nitrogen oxide responsible for regulatory functions, including those associated with antioxidant activity [4, 7, 11].

Study of biochemical indicators in the population of the observation group (table 3), associated with these genes, confirms the importance of the interaction that occurs between environmental factors and features of genetic polymorphism when it comes to resulting phenotypic manifestations. Thus, the population covered showed reduced concentrations of nitrogen oxide in serum in 66.7% of cases relative to the values of the comparison group, on average of 1.2 times ($p<0.05$). The level of superoxide dismutase in the examined groups did not differ significantly.

Summing up what has been said, genetic analysis revealed a significant ($p=0.02$) increase in the frequency of occurrence of the minor allele of eNOS rs1799983 gene associated with a decrease in serum levels of nitrogen oxide, which proves the formation of additional risk factors under technogenic influence.

Conclusions. When studying the population living in the Southern Siberia regions under intensive technogenic impact, significant changes in immune regulatory indicators were observed. What is more, there was a ratio violation of the main populations of immunocompetent cells with a decrease in the fraction of $CD16^+CD56^+$-, $CD3^+$- and $CD4^+$-cells, the expression of the activation marker CD25, a decrease in the production of serum immunoglobulins IgG and IgM combined with the development of sensitization with an increase in the production of specific IgG antibodies to aluminum, benz(a)pyrene, IgE to formaldehyde. When sensitivity of the population increased, the features of genetic polymorphism of the eNOS gene associated with a decrease in serum levels of nitrogen oxide were revealed, which indicates additional risk factors associated with the development of inflammatory and oxidative reactions, disorders in formation of the optimal adaptive oxygen-dependent processes in the body that provide detoxification and protection of endothelial cells.

The revealed features of the indicator immunogenetic indicators in the population on the surveyed area are proposed to be used as markers of pathological health disorders for finding solutions to treatment and preventive tasks in emerging technogenic geochemical provinces.

Table 2

Features of genetic polymorphism in the population under technogenic exposure

Gene (polymorphism)	Genotype, allele	Observation group, %	Comparison group, %	χ^2	p
eNOS (rs1799983)	GG	65.8	87.1	4.68	0.1
	GT	28.9	12.9		
	TT	5.3	0		
	G	80.3	93.5	5.08	0.02
	T	19.7	6.5		
SOD2 (rs2758330)	CC	60.5	67.7	0.42	0.81
	CA	28.9	22.6		
	AA	10.5	9.7		
	C	75	79	0.31	0.58
	A	25	21		

Table 3

The biochemical markers in the population under technogenic exposure

Index	Reference interval	Observation group	Comparison group
Nitric oxide, μmol/dm^3	70.4-208.6	108.69±16.103*	134.39±17.304
Superoxide dismutase, ng/cm^3	30.1-88.1	61.067±11.316	65.679±11.201

Note: * - the significance of differences relative to the comparison group ($p<0.05$).

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COMPARATIVE EXPERIMENTAL STUDY OF THE PERFORMANCE OF BACTERIOSCOPIC METHODS IN DETECTING ACID-FAST BACILLI: ZIEHL-NEELSEN MICROSCOPY, CONVENTIONAL FLUORESCENCE, AND LED FLUORESCENCE MICROSCOPY

ABSTRACT

Performance of bacterioscopic methods was comparatively studied in Bacteriologic Laboratory of the Phthisiatry Research-Practice Center, to assess the detection of acid-fast bacilli (AFB) by Ziehl-Neelsen (ZN) microscopy, conventional fluorescence microscopy (FM), and LED fluorescence microscopy (LED-FM). A total of 400 positive smears detected by FM were re-observed by ZN microscopy; additionally, we analyzed diagnostic material from 648 patients of the Pulmonology Department of the Yakutsk City Clinical Hospital, in whom the hospital laboratory did not detect