

Some Aspects of Immune Status of Primary and Secondary Schoolchildren Living in Conditions of Technogenic Pollution of Urban Environment by Industrial Pollutants

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ABSTRACT

Under conditions of intensive industrial impact, the study of the specifics of the regulatory systems response will benefit effective implementation of measures aimed to preserve the health of the child population. We have studied the indicators of the immune status of schoolchildren at different levels of education under the industrial impact of the urban environment. CD-populations of lymphocytes have been studied by flow cytometry. Humoral immunity has been assessed based on the production of serum immunoglobulins by radial immunodiffusion, and specific antibodies to chemical factors – by allergosorbent testing. It has been revealed that the immune profile imbalance is especially pronounced in middle school students and is characterised by the following: a decrease in CD19⁺ and CD8⁺ lymphocytes, a reduced phagocytic activity, an increase in IgM and IgA production, a high level of sensitization for specific IgE to manganese, nickel, formaldehyde, IgG to lead, benzene, phenol, changes in the cytokine markers of interleukin-4 and interferon-gamma. These indicators reflect the state of immunological health of schoolchildren and serve as indicators of the formation of pathological trends in the context of industrial changes of the environment, which can be used for early detection of immune-mediated morbidity and development of effective preventive measures to improve the quality of life and provide a decrease in the incidence rate of children.

Keywords: immune status, primary and secondary schoolchildren, technogenic pollution, urban environment, industrial impact, regulatory systems response

1. INTRODUCTION

The scale of technogenic impact on the environment is constantly increasing, the problem of the quality of living conditions is especially important in industrially developed regions where constant monitoring of both the state of the environment and the influence of the external environment on the health of the population with regards to the impact specifics and the peculiarities of the adaptive reserve of the regulatory systems of the body including immune protection is required [1–5].

Preserving the health of the younger generation in conditions of intensive industrial exposure is possible through the implementation of complex measures

regarding the age characteristics of schoolchildren and based on the development of prognostic criteria for the functional state of the immune regulation system aimed to ensure early detection of immune-mediated morbidity and improve the effectiveness of treatment and prophylactic measures [6–8].

Violation of the harmonious physiological development of children and adolescents is associated with an unfavorable combination of technogenic chemical, physical and socio-economic factors, which under conditions of increased stress loads form the insufficiency of regulatory systems for ensuring homeostasis and serve as a prerequisite for the

development of immune-mediated diseases. The immune system reacts to environmental factors by functional shifts of both the cellular and humoral defense links, which, as a result of excessive loads, can contribute to the formation of disorders with pathological changes in the state of health, the identification of which at early stages will provide a decrease in the incidence rate of the child population in conditions of unfavorable changes in the environment [9–11].

The purpose of the study is to determine the peculiarities of changes in the parameters of immune regulation in primary and secondary schoolchildren living in areas with an intensive level of industrial exposure.

2. MATERIALS AND METHODS

Schoolchildren living in a large industrial center with a developed industrial infrastructure were involved in the study. The state of health of children of the 1st grade (observation group No.1 comprising 48 children with an average age of 7.36 ± 0.07 years) and 6th grade children (observation group No. 2 comprising 27 children with an average age of 12.81 ± 0.13 years) was studied. The comparison groups consisted of schoolchildren of the 1st grade (comparison group No.1 children with an average age of 7.58 ± 0.10 years) and 6th grade (comparison group No.2 comprising 35 children with an average age of 12.47 ± 0.10 years) studying at a general education institution of the regional center with no large industrial enterprises. All groups were comparable in terms of age, gender, presence of somatic diseases ($p > 0.05$).

All legal representatives of the children signed the informed consents to participate in the study. The work was carried out in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Federal Scientific Research Center for Medical and Preventive Technologies for Managing Public Health Risks.

The content of metals (manganese, nickel, and lead) in biological media of children was determined on an Agilent 7500cx mass spectrometer (Agilent Technologies Inc., USA) by the inductively coupled plasma method in accordance with the organization's standards and guidelines. The level of organic compounds (benzene, phenol) was investigated by gas chromatography on a gas chromatograph with a flame ionization detector Kristall 5000 (Russia); formaldehyde was studied on an Agilent 1200 chromatograph by high performance liquid chromatography.

To assess the immune status of schoolchildren, cell parameters were studied using membrane CD markers; lymphocyte fractions were determined on a FACSCalibur flow cytometer (Becton Dickinson, USA)

using the universal CellQuest.PrO program; panels of labeled monoclonal antibodies were used, and at least 10000 events were recorded in total. Phagocytic activity was studied using formalized sheep erythrocytes as objects of phagocytosis. Serum immunoglobulins IgA, IgM, IgG were determined by the method of radial immunodiffusion according to Mancini, and the content of total IgE, interleukin-4, interferon-gamma – on the analyzer Elx808IU (BioTek, USA) by enzyme immunoassay. To identify specific IgE antibodies to manganese, nickel, formaldehyde, IgG to lead, benzene, phenol, we used allergosorbent testing with an enzyme label.

The performed chemical-analytical and immunological studies were carried out on certified equipment according to standard methods in accredited laboratories of the Federal Scientific Research Center for Medical and Preventive Technologies for Managing Public Health Risks.

Statistical analysis of the data was performed using the Statistica 6.0 software package (Statsoft, USA); arithmetic mean (M) and standard error of the mean (m) were calculated. The significance of intergroup differences was assessed by the Student's t-test; the differences were considered significant at $p < 0.05$.

3. RESULTS

According to the results of chemical analysis (Table 1), schoolchildren of observation group No. 1 permanently residing in conditions of intense industrial exposure showed high concentrations of chemicals in the blood, exceeding the background levels established for the region (Perm region of Russia) in 62.2 % of children for nickel, 47.6 % for benzene, 97.8 % formaldehyde ($p=0.000-0.001$). For observation group No.2, a significant excess of the background level was also shown for nickel, benzene, formaldehyde in 88.9 %, 46.2 %, 100 % of children, respectively ($p=0.000-0.006$).

Immunodiagnostic examination of children showed pronounced changes in cellular parameters, production of immunoglobulins and specific sensitization of schoolchildren in the observation group (Table 2). The features of the CD immunogram did not significantly differ from the physiological level. However, for the observation group No. 2, there was predominantly 1.47 times decrease in CD19⁺ cells relative to the comparison group ($p=0.016$), as well as 1.15 times increase in the number of CD3⁺ lymphocytes ($p=0.049$, the differences are reliable in terms of the frequency excess of the norm). Depressing effects in the observation group No.2 were noted for the fraction of CD8⁺ lymphocytes, on average by 1.33 times, while a significant shift of 1.36 times in the CD4⁺/CD8⁺ parameter was shown ($p=0.001-0.03$).

When assessing phagocytic activity, no deviation of indicators from the physiological norm was revealed, however, a decrease in the level of phagocytosis by the value of relative phagocytosis and phagocytic number in the observation group No. 2 relative to the comparison group was shown by 12 and 14 %, respectively ($p=0.001-0.003$).

The levels of serum immunoglobulins of classes A, M and G in the examined schoolchildren corresponded to the age norm, at the same time, a 16.4 % IgG overproduction was revealed in the observation group No.1 relative to the comparison group No.1 ($p=0.000$) and IgM ($p=0.044$, the differences are significant by the multiplicity of norm exceedance). The schoolchildren of the observation group No.2 also showed a tendency to the activated production of immunoglobulins with a significant value according to the IgM criterion ($p=0.043$, the differences are significant in the multiplicity of norm exceedance) and IgA ($p=0.018$) by 8.8 and 22.9 %, respectively, relative to the comparison group No.2. When comparing the indicators of schoolchildren at different levels of education, there was revealed an excess of IgM and IgA levels in the observation group No.2 by 1.2 and 1.31 times, respectively ($p=0.001-0.006$).

At the same time, in the observation group No.2, an increase in the level of general sensitization according to the IgE criterion was noted in 2.65 times relative to the comparison group No. 2, and also 2.71 times relative to the observation group No.1 ($p=0.047-0.049$). An increase in the level of specific immunoglobulins IgG to benzene, phenol, lead in comparison with the reference values was shown in 70.8–88.9 % of children in the observation groups ($p=0.000$). In addition, the content of specific IgE antibodies to manganese, nickel, formaldehyde, IgG to lead, benzene, phenol in the observation group No.1 exceeded the indices of the comparison group by 7.7, 8.4, 9.7, 4.8, 5.1 and 6.7 times, respectively ($p=0.000$). In the observation group No.2, an increase in these indicators was also noted by 5.3, 10.6, 12.1, 4.8, 6.9, 6.0 times, respectively ($p=0.000$).

Analysis of the cytokine regulation mechanism indicated multidirectional trends in the production of regulatory markers associated with an average 3.39 times increase in the level of interleukin-4 significant in the observation group No. 1 relative to the comparison group, and inhibition of interferon-gamma in the observation group No.1 by 2.22 times and in the observation group No.2 by 2.15 times ($p=0.000$). When comparing the indicators of the observation groups No. 1 and No. 2, a significant decrease in the level of interleukin-10 was noted on average by 1.51 times ($p=0.000$).

The study indicates significant changes in the immune system of schoolchildren in living conditions with a high industrial load, and for students of the middle stage of education, more significant changes in immune reactivity are characteristic, which indicates the significant importance of both the specificity of the external environment and the duration of exposure, and the age characteristics of children [12, 13]. Numerous scientific works confirm the relevance of studying the characteristics of the response of the immune regulation system in conditions of unfavorable environmental influences among different age groups of the population, children are especially sensitive to changes in living conditions, which is associated with imperfect adaptive mechanisms and, with an increased contaminant load, can determine the formation of a high level of morbidity in areas of ecological trouble [14–16]. The changes in the immune status of middle school students revealed in this work reflect not only functional shifts in immunocompetent cells associated with hyperproduction of immunoglobulins, but also a shift in the balance of cell subpopulations. Especially noteworthy is the high level of specific sensitization of pupils and, in general, changes in the humoral component of the immune adaptation mechanism for an effective response and preventive measures to improve the quality of life and preserve the health of the child population.

Table 1. The content of contaminants in the blood of the examined schoolchildren under industrial impact (M±m)

Indicator	Background level	Observation group No.1	Observation group No.2
Manganese, $\mu\text{g}/\text{cm}^3$	0.013±0.004	0.011±0.001	0.012±0.002
Nickel, $\mu\text{g}/\text{cm}^3$	0.0022±0.002	0.0053±0.00164a	0.0064±0.00223a
Lead, $\mu\text{g}/\text{cm}^3$	0.0144±0.067	0.0111±0.00139	0.0117±0.00202
Formaldehyde, $\mu\text{g}/\text{cm}^3$	0.005±0.0076	0.035±0.00537a	0.0343±0.00427a
Benzene, $\mu\text{g}/\text{cm}^3$	0.0±0.0	0.00028±0.00015a	0.00023±0.00017a
Phenol, $\mu\text{g}/\text{cm}^3$	0.01±0.0369	0.00645±0.00159	0.00559±0.00151

Note: a) differences between the observation groups No. 1 and No. 2 and the background level to Perm region of Russia are significant ($p < 0.05$).

Table 2. Change in immune parameters and specific sensitization in schoolchildren under industrial impact (M±m)

Indicator	Observation group No.1	Observation group No.2	Comparison group No.1	Comparison group No.2
CD16 ⁺ CD56 ⁺ -lymphocytes, 10 ⁹ /cm ³	0.43±0.16	0.36±0.11	0.32±0.04	0.39±0.075
CD16 ⁺ CD56 ⁺ - lymphocytes, %	16.2±4.91	17.0±6.85	13.8±2.02	16.8±2.887
CD19 ⁺ - lymphocytes, 10 ⁹ /cm ³	0.34±0.14	0.27±0.08 ^b	0.3±0.048	0.397±0.068
CD19 ⁺ - lymphocytes, %	11.8±2.78	11.9±2.91 ^b	12.53±1.25	17.47±3.51
CD3 ⁺ - lymphocytes, 10 ⁹ /cm ³	1.86±0.39	1.56±0.43	1.68±0.23	1.39±0.30
CD3 ⁺ - lymphocytes, %	66.8±5.26	67.5±6.60	69.67±2.36	58.47±8.15
CD3 ⁺ CD4 ⁺ -lymphocytes, 10 ⁹ /cm ³	1.05±0.29	0.89±0.28	0.92±0.15	0.87±0.14
CD3 ⁺ CD4 ⁺ - lymphocytes, %	37.3±4.56	38.3±5.91	37.8±2.62	37.13±3.36
CD3 ⁺ CD8 ⁺ -lymphocytes, 10 ⁹ /cm ³	0.62±0.109	0.56±0.14 ^b	0.606±0.083	0.75±0.11
CD3 ⁺ CD8 ⁺ - lymphocytes, %	22.8±3.16	24.3±3.2 ^b	25.4±2.13	32.4±3.27
CD4 ⁺ /CD8 ⁺ , c.u.	1.7±0.35	1.63±0.30 ^b	1.53±0.17	1.20±0.22
Absolute phagocytosis, 10 ⁹ /dm ³	1.78±0.254	2.16±0.395	1.97±0.306	1.99±0.198
Percentage of phagocytosis, %	49.54±3.65	48.11±2.79 ^b	53.0±3.16	54.8±2.38
Phagocytic number, c.u.	0.94±0.083	0.907±0.08 ^b	1.03±0.094	1.08±0.077
Phagocytic index, c.u.	1.89±0.058	1.871±0.083	1.92±0.068	1.94±0.065
IgG, g/dm ³	11.76±0.53 ^a	12.67±1.09	10.10±0.64	11.92±0.71
IgM, g/dm ³	1.495±0.10 ^c	1.74±0.14	1.48±0.08	1.60±0.13
IgA, g/dm ³	1.47±0.13 ^c	1.93±0.23 ^b	1.53±0.14	1.57±0.19
Total IgE, ME/cm ³	96.14±54.80 ^c	260.29±157.26 ^b	131.68±89.87	98.31±51.54
IgE manganese specific, ME/cm ³	0.34±0.08 ^a	0.3±0.13 ^b	0.044±0.007	0.057±0.009
IgE nickel specific, ME/cm ³	0.37±0.07 ^a	0.50±0.17 ^b	0.044±0.009	0.046±0.008
IgE formaldehyde specific, ME/cm ³	0.31±0.05 ^a	0.35±0.12 ^b	0.032±0.006	0.029±0.005
IgG benzene specific, c.u.	0.32±0.06 ^a	0.31±0.07 ^b	0.067±0.016	0.065±0.015
IgG phenol specific, c.u.	0.29±0.05 ^a	0.33±0.06 ^b	0.057±0.009	0.048±0.01
IgG lead specific, c.u.	0.34±0.06 ^a	0.37±0.08 ^b	0.051±0.009	0.062±0.009
Interleukin-4, pg/cm ³	1.56±0.40 ^a	0.97±0.50	0.46±0.25	0.46±0.15
Interferon - gamma, pg/cm ³	2.29±0.38 ^a	2.27±0.47 ^b	5.08±0.79	4.87±0.58
Interleukin-10, pg/cm ³	2.77±0.52 ^c	4.81±0.69	4.17±2.19	3.83±1.69

Note: a) differences between the observation group No. 1 and the indices of the comparison group No. 1 are significant (p <0.05). b) the differences between the observation group No. 2 and the indices of the comparison group No. 2 are significant (p <0.05). c) the differences between the observation group No. 1 and the indices of the observation group No. 2 are significant (p <0.05).

4. CONCLUSION

The present study of the immune profile of schoolchildren living in areas with a high intensity of industrial exposure showed significant changes in both cellular and humoral components of immune reactivity (especially pronounced for middle school pupils) associated with a change in the ratio of CD4+/CD8+ lymphocytes, phagocytic activity, cytokine markers, production of serum immunoglobulins IgA and IgM and an increase in the level of specific sensitization to industrial pollutants, which can be used to solve the problems of monitoring the health status of different age groups of schoolchildren in conditions of different intensity and nature of exposure to environmental factors.

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