IMMUNOLOGIC STUDY OF WORKERS ENGAGED IN ANTIMONY PRODUCTION

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SUMMARY: Immune status of 1756 persons was evaluated using immunologic level 1 and 2 tests. The extent of immunologic effects of antimony is dependent on the level of antimony exposure received by the body. At low levels of antimony exposure compensatory activation of some immune parameters is noted. The continual exposure to high antimony levels induces immune deficiency states which in their first stages are characterized by impairment of T cell immunoregulatory function. Based on the degree of immunodeficiency manifestations, subjects were divided into a primary risk and an increased risk group for immunodeficiency. 23.49% of workers were included in the primary risk group and 3.62% - in the increased risk group. The main clinical signs of immunodeficiency were infectious and allergic syndromes. Characteristics of immune parameters in workers of both risk groups show an imbalance of immune indices, low level of adaptation, evidence of sustained immune deficits and development of secondary immunodeficiency. A relationship of immune parameters to the length of service at the antimony integrated plants was found. There was a compensatory activation of immune function during the first 5 years of work. This was followed by successive stages of slight, moderate and severe degree of immunodeficiency after 5-7, 10-12 and 15 or more years of service at the plant, respectively.

Key Words: Antimony, antimony production, immunodeficiency.

INTRODUCTION

The rapid scientific and technological progress is accompanied by an intensive environmental pollution all over the world. In this situation, the human body is being exposed to an increasing variety of physico-chemical substances which exert a significant influence on the immune system and often cause immunopathological conditions (1-3). Industrialization processes in developed countries have involved a number of heavy metals (mercury, lead, cadmium, antimony, etc.) which are discharged in great amounts into the biosphere and pose a serious threat to the health of the people, especially those occupationally exposed to them. Therefore, the assessment of immune status of different occupational populations with the subsequent development of methods for their immunorehabilitation is one of the urgent tasks of modern immunology. Performing immunologic examinations and detecting specific

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immune abnormalities will allow immunodeficiency risk groups to be identified, which will help in the providing a specialized care to these groups including targeted immune correction therapy.

The richest deposits of antimony ore are concentrated in the territory of the Kyrgyz Republic where in the settlement of Kadamzhai an integrated antimony plant operates with the production of standard pure antimony. Antimony is one of the major environmental pollutants placed by WHO experts in the category 1 of toxic substances hazardous to health and the biological resources of the World Ocean. However, the demand for antimony is steadily growing in all industrialized regions in the world. The amount of antimony produced annually in the world is 150 times that naturally released into the biosphere. Thus, the main cause of antimony environmental pollution and its adverse effect on human health is the economic activity of people themselves.

The purpose of this study was to investigate the main indices of T and B cell immunity and the system of mononuclear phagocytes in the workers of the Kadamzhai antimony integrated plant.

MATERIALS AND METHODS

Studies were performed in the Kadamzhai antimony integrated plant, at the Kadamzhai District of the Osh Region of the Kyrgyz Republic, and in Fergana Region of the Uzbek Republic. Control subjects were chosen from the Chui Valley and in some cases from the Kadamzhai District. The immunologic evaluation was done according to the current 3-stage scheme for screening large populations: prescriptive screening with a diagnostic questionnaire developed by the Institute of Immunology of the Russian Federation Ministry of Health, Immunologic studies using a complex of level 1 immunologic microassays and the establishing of a group of primary and a group of increased risk of immunodeficiency and other immunopathological states on the basis of the results of the 2 prior stages (4,5).

The number of T and B lymphocytes was determined by sheep and mouse red cell E rosette formation tests respectively, T lympocyte subpopulations (T helper, suppressor cells) were assessed by theophylline sensitivity, B lymphocyte function was assessed by the IgA, IgM and IgG synthesis, phagocytic activity was judged by neutrophil ingestion of latex particles.

The following more sophisticated immunologic tests were performed. The functional activity of T lymphocytes was assessed by the blastogenesis test with phytohemaglutinin according to S. Frankenburg (6) and the blastogenesis test using whole blood according to the commonly used method (7). Circulating T and B lymphocytes, T helper and suppressor cells were determined using monoclonal antibodies ICO-87, ICO-12, ICO-86 and ICO-31, respectively. Monocyte phagocytic activity was assessed in a monodisperse latex particle test (3). Bactericidal oxygen dependent systems were assayed by the nitroblue tetrazolium reduction test (8) which correlates with the superoxide radical producing activity of phagocytes. Diformazan positive cells were measured and a mean reaction cytochemical coefficient was calculated (9). Lysosome content in monocytes was estimated by the total luminescence index of lysosomes (5). Monocyte adhesiveness and spreading was determined according to the method of L. F. Bluger *et al.* (1) as modified by I. S. Freudlin (5). Fc and C3 expression was assessed in EA and EAC monocyte rosette formation tests (3).

These immunologic studies were performed in 268 apparently healthy residents of the Kadamzhai settlement, 143 persons from the city of Bishkek and 1345 workers from the Kadamzhai antimony integrated plant. To assess antimony accumulation in the body antimony was determined in hair by the atomic absorption spectrophotometric technique on a mercury analyzer "Yulia-2" (Russia). Statistical analysis was made using a computer "Iskra-1030" (Russia). Based on the correlational analysis, correlation pleiads were constructed.

RESULTS AND DISCUSSION

The results of the immunologic evaluation of the Kadamzhai plant's workers are shown in Table 1, from which it can be seen that with increasing length of service at the antimony plant secondary immunodeficiencies of combined type develop and increase. 23.49% of workers (n = 397) were ascribed to the primary risk group for immunodeficiency and 3.62% (n = 52) - to the increased risk group for immunodeficiency. The distribution of immunedeficiency syndromes by their rate in workers is presented in Table 2. As can be seen from it, infectious syndrome and combined infectious-autoimmune syndrome are the most prevalent. Furthermore, while in risk group 1 allergic and autoimmune syndromes still occur as isolated states, in risk group 2 they are seen only in conjunction with infectious syndrome.

In risk group 1 workers the absolute and relative number of T lymphocytes was decreased as compared to controls and the population of the settlement

Table 1: Immune variables in different groups of population

Variables	Controls		Antimony plant workers		
	Population of Bishkek	Population of Kadamzhai settlement	Apparently healthy workers	Immunodeficiencey resk group 1	Immunodeficiency risk group 2
	n=143	n=268	n=896	n=397	n=52
Antimony content in hair, mcg/g	0.8	3-10	5-15	10-30	50-80
T lymphocytes, %	63.4 ± 2.3	49.7 ± 0.74	42.8 ± 3.88	39.5 ± 0.58^{xo}	33.7 ± 2.7^{xo}
T lymphocytes thous, per mcl	1117 ± 40.5	1098 ± 30.6	1233 ± 28.8^{o}	911.5 ± 32.3^{xo}	649.8 ± 29^{xo}
Theoph. resist. E-RFC, %	40.9 ± 0.9	33.6 ± 1.1^{x}	38.0 ± 1.5^{o}	30.4 ± 1.25^{x}	23.2 ± 1.8^{x}
Theoph. sensit. E-RFC, %	12.2 ± 0.6	16.6 ± 0.7^{x}	18.2 ± 1.0^{x}	13.3 ± 0.4^{0}	19.9 ± 1.2^{xo}
B-lymphocytes, %	17.7 ± 0.3	18.8 ± 0.4	20.1 ± 2.1	16.3 ± 0.3^{o}	11.4 ± 1.2^{xo}
B-lymphocytes thous. per mcl	422.5 ± 24.9	411.2 ± 14	466.1 ± 14.0^{o}	388 ± 12.0	198.8 ± 13.1^{x}
IgM, g/l	0.87 ± 0.04	1.25 ± 0.06	1.38 ± 0.08	0.64 ± 0.05	0.45 ± 0.02
IgG, g/l	15.7 ± 0.05	21.7 ± 0.4	19.9 ± 0.37^{x}	18.5 ± 0.08	20.5 ± 0.03
IgA, g/l	0.82 ± 0.03	1.60 ± 0.06	1.34 ± 0.05	0.47 ± 0.02	0.41 ± 0.02
Phagocytic index	72.7 ± 2.6	61.9 ± 0.8	61.2 ± 0.7	66.1 ± 3.8	36 ± 1.5^{x}
Phagocytic number, latex per neutrophil	6.97 ± 1.3	5.7 ± 0.9	6.2 ± 0.4	4.74 ± 2.8	1.32 ± 1.3^{x}

Note: x - Significantly different as compared with controls from Bishkek;
o - Significantly different as compared with controls from the Kadamzhai District (P < 0.05);
theoph. resist. (sensit.) E-RFC - theophylline resistant (sensitive) E rosette forming cells.

(P<0.05). The percentage of theophylline sensitive and B lymphocytes was decreased as compared to the population of the settlement (P<0.005). The amount of monocytes in blood was diminished as well as their function in such tests as nitroblue tetrazolium test, adhesion and spreading, C3 and Fc receptor expression (P<0.05).

In risk group 2 workers, the abnormality of these parameters was still greater. Compared to controls and the population of the settlement they had lower T and B lymphocyte numbers, lower theophylline resistant cells and higher theophylline sensitive cells (P<0.05), depressed phagocytosis indices, an imbalance of serum immunoglobulins with IgM and IgA being

Table 2: Distribution of clinical syndromes of immunodeficiency in antimony plant workers.

Clinical syndromes of immunodeficiency	Immunodeficiency risk group 1, %	Immunodeficiency risk group 2, %
Infectious syndrome	57.3	27.7
Allergic syndrome	7.86	0
Autoimmune syndrome	1.69	0
Lymphoproliferative syndrome	0	0
Infectious-allergic syndrome	16.3	29.64
Infectious-autoimmune syndrome	10.7	20.4
Allergic-autoimmune syndrome	1.96	0
Infectious-allergic-auto-immune syndrome	4.21	22.22

decreased and IgG relatively increased. In addition, the migration and blast-transformation capacity of leukocytes diminished with increasing length of service.

A surge of some immune parameters (theophylline sensitive E rosette forming lymphocytes, IgM, absolute number of T lymphocytes) was observed in the group of apparently healthy workers of the antimony plant.

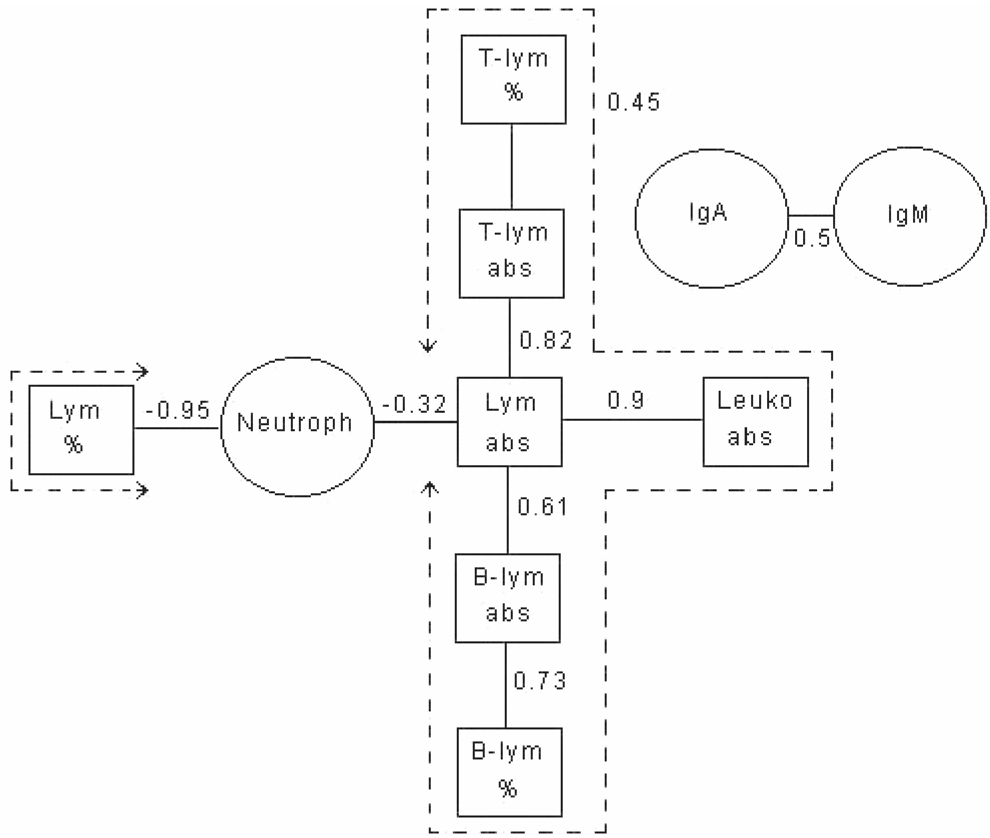
What can these findings point to? The interesting features found in the immune variables of various groups of workers can be interpreted, in our opinion, in the following way: first, mean immune parameters are higher in the population of the biogeochemical region than in the population of Bishkek which indicates that Kadamzhai residents are exposed to toxic materials. This feature is explained by a known fact that low level toxic exposure causes nonspecific activation of some immune parameters. Secondly, the exposure to antimony received by persons living in the antimony region is redoubled when they take up a job at the plant. Under these conditions the immune system is destabilized and goes through a period of "adaptation storms". Thirdly, in the risk group 1 the adaptive process begins to be exhausted due to antimony accumulation in the body. In the risk group 2 the immune system, however, attains a certain stabilization but at a lower level of adaptation with development of sustained immune deficits and chronic infections.

Table 3: Percentages of T and B lymphocytes and their subpopulations determined using monoclonal antibodies in the studied groups.

	Controls	Population of Kadamzhai District	Auxiliary professions	Main professions
	n=15	n=23	n=17	n=78
T lymphocytes	56.6 ± 3.7	53.0 ± 3.5	30.0 ± 3.5^{xo}	29.0 ± 3.0^{xo}
T helper cells	39.0 ± 4.2	24.0 ± 3.2^{x}	15.0 ± 2.6^{xo}	20.0 ± 2.6^{xo}
T suppressor cells	15.0 ± 3.2	17.0 ± 2.2	12.0 ± 3.1	14.0 ± 3.1
B lymphocytes	30.0 ± 4.5	17.0 ± 2.2^{x}	15.0 ± 2.2^{x}	20.0 ± 3.3

Note: x - Significantly different as compared with controls.
o - Significantly different as compared with the population of the Kadamzhai District.

Figure 1: Correlational pleiad of immune indices in the population of the city of Bishkek.



To corroborate the above mentioned observations we investigated associations among various immune parameters within study groups by constructing correlational pleiads that reflected the most consistent associations between immune parameters. (Figures 1, 2, 3, 4, 5 and 6).

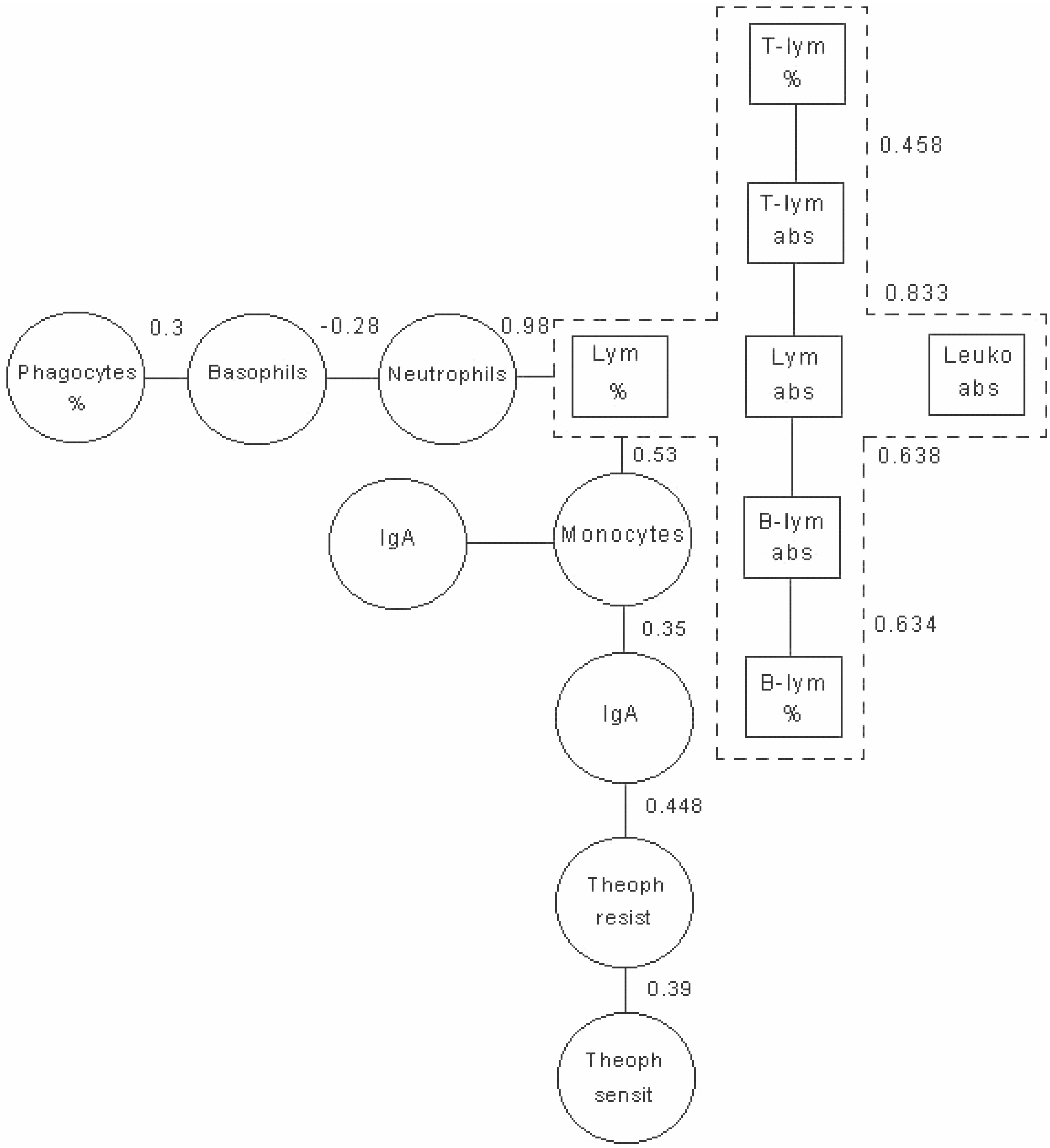
The correlational analysis showed a high negative association of lymphocyte absolute and relative count with neutrophil count, which agrees well with the concepts of the adaptive process in the body. With increasing antimony exposure of the body there occur switches in associations between these three parameters and coefficients may change their signs to positive ones. Monocytes and basophils can also switch from negative associations with neutrophils to positive ones with the relative number of lymphocytes in the group of apparently healthy workers and even with the relative number of T lymphocytes.

The behaviour of eosinophils is noteworthy. While in residents of Bishkek and the settlement of Kadamzhai they show correlative relationships with basophils and neutrophils, in apparently healthy workers and immunodeficiency risk group 1 and 2 workers they switch to a relationship to T lymphocytes. Clinically, this is supported by the presence of allergodermatitis due to metallic antimony dust in a part of workers.

All the above mentioned changes indicate that a period of "adaptation storms" is experienced by apparently healthy workers and a stabilization of immune function takes place in workers of immunodeficiency risk groups 1 and 2 though on a lower level of reactivity that does not provide protection against infection.

The correlational analysis revealed in all pleiads the presence of a core of persistent associations in the form of a cross. Some elements of the core structure may break away from the core changing places with their substitutes (3). This indicates the destabilization of immune function. Thus, by a mathematical means it

Figure 2: Correlational pleiad of immune indices in the Kadamzhai settlement.



has been possible to bring out a readjustment process in the immune system which is trying to find a new optimum of functioning in response to antimony exposure.

We were interested in tracing alterations indicating changes in immunity with respect to the length of service at the antimony plant which was the next stage of our study. At first, mean values of immune parameters were investigated in various professional groups of workers versus the population of Kadamzhai (Table 3). As seen from the table, workers of auxiliary and main professions show decreased T lymphocytes and T helper cells, workers of auxiliary professions show decreased B lymphocytes ($P < 0.05$). T suppressor cells and B lymphocytes are at the level of control values in the main professions. In all professional

Table 4: Percentages of T, B lymphocytes and their subpopulations in workers by profession.

	Controls	Kadamzhai Population	Operatives	Founders	Other main Professions	Underground Workers
	n=15	n=23	n=22	n=23	n=21	n=7
T lymphocytes	56 ± 3.7	53 ± 3.5	27 ± 2.5^{xo}	30 ± 2.4^{xo}	27 ± 3.2^{xo}	30 ± 3.6^{xo}
T helper cells	39 ± 4.2	24 ± 3.2^{x}	17 ± 2.9^{x}	18 ± 1.0^{x}	17 ± 2.7^{x}	26 ± 3.2^{xx}
T suppressor cells	15 ± 3.2	17 ± 2.2	12 ± 1.8	9 ± 3.0^{o}	11 ± 2.9	16 ± 5.1
B lymphocytes	30 ± 4.5	17 ± 3.2	17 ± 2.4	23 ± 3.2	14 ± 4.7	21 ± 2.4

Note: x - Significantly different as compared with controls.
o - Significantly different as compared with the population of Kadamzhai.
xx - Significantly different as compared with other professions.

groups (Table 4) T lymphocytes, T helper cells and B lymphocytes were found to be in decreased numbers ($P < 0.05$). It should be noted that T helper cell count is significantly higher in underground versus surface workers ($P < 0.05$) though there is no difference between underground workers and controls.

The analysis of changes in immune parameters in relation to the length of service showed a steady deterioration of immune parameters with increasing duration of occupational exposure to antimony at two main departments of antimony production: pyrometallurgical (founders) and hydrometallurgical (operatives). Slight,

Figure 3: Correlational pleiad of immune indices in apparently healthy workers of the antimony plant.

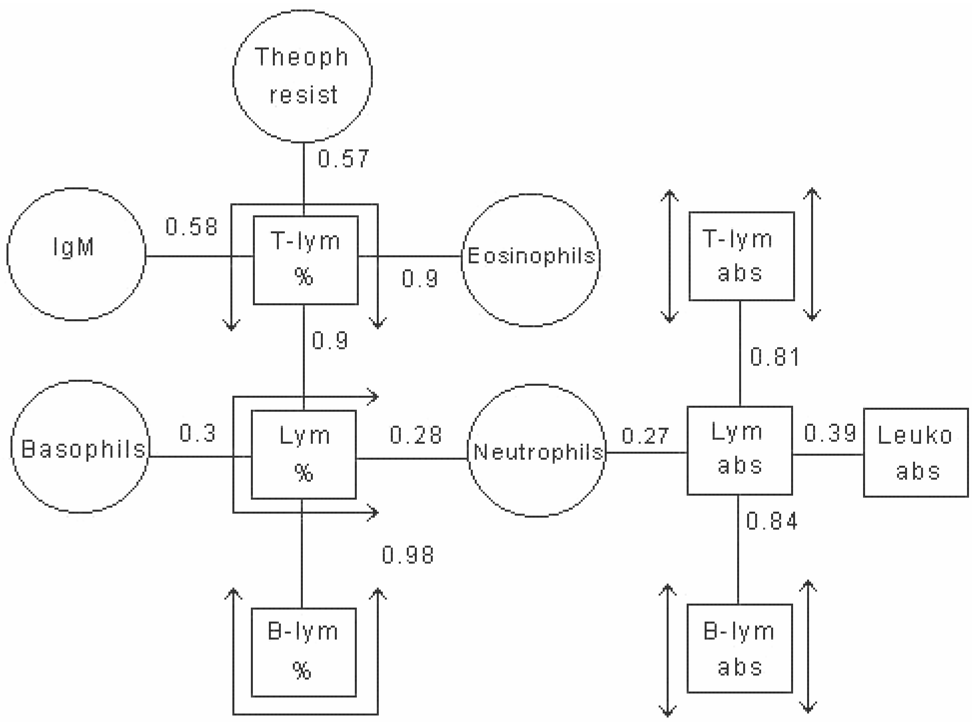
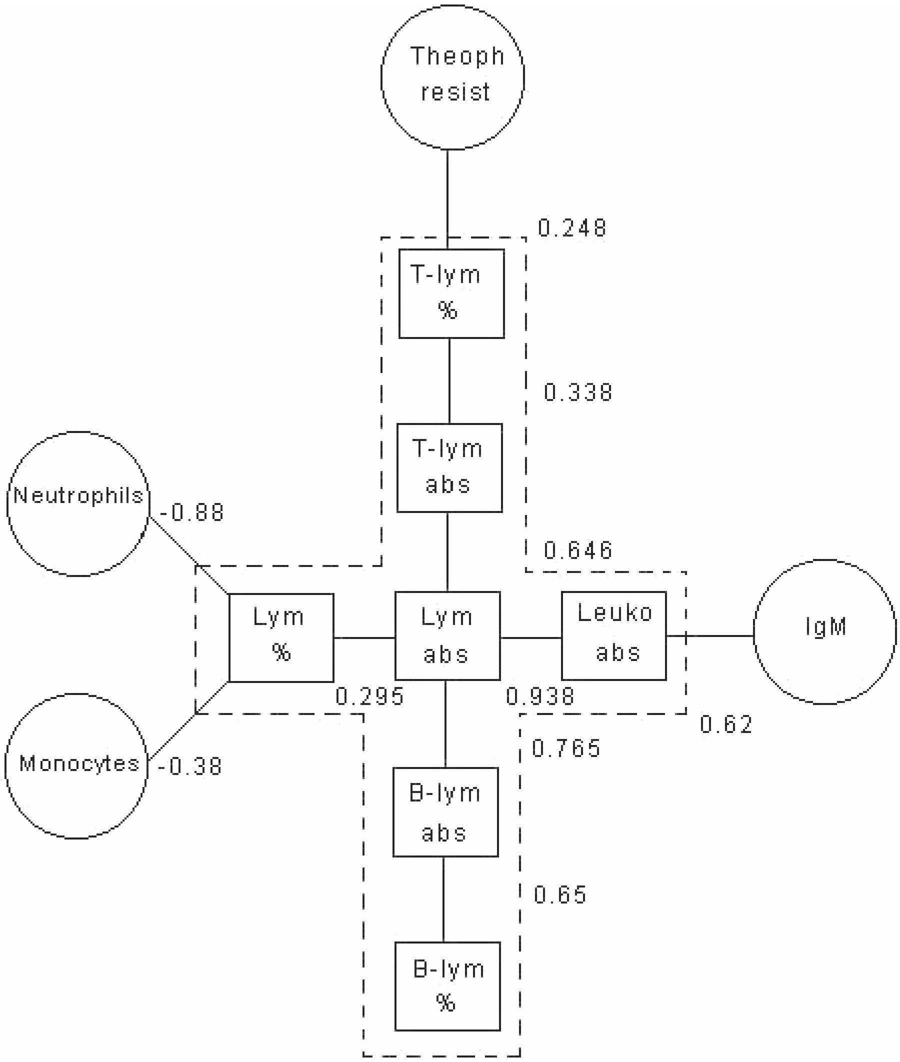


Figure 4: Correlational pleiad of immune indices in workers of the immunodeficiency risk group 1.

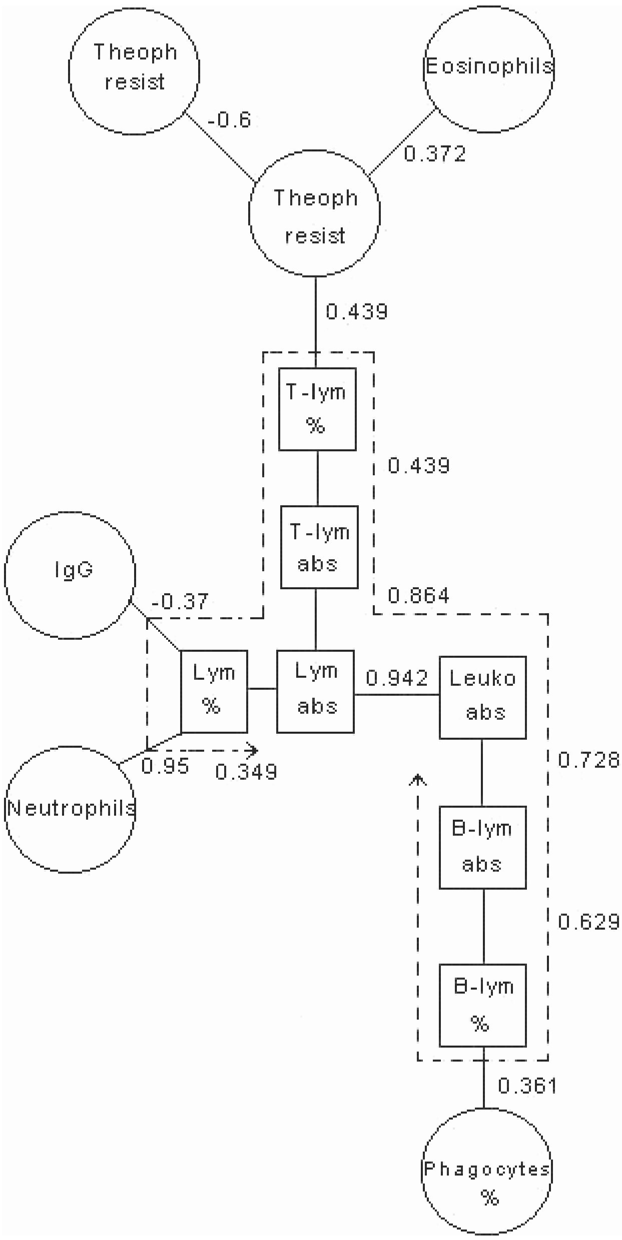


moderate and severe variants of immunodeficiency were identified in them.

1. After 5-9 years of employment, a significant reduction in the total number and function of T lymphocytes, primarily in the number of T helper cells, begins to occur but is of transient character. The proportion of B lymphocytes is within normal limits (18.0 ± 0.9%) but their antibody producing function is enhanced. However, an imbalance of serum immunoglobulins is observed which consists in decreased levels of IgM (1.05 ± 0.1 g/l) and IgA (0.9 ± 0.07 g/l) and normal levels of IgG (19.4 ± 0.08 g/l). Doubtless, the state of plasma outer membrane of mononuclear phagocytes is their important functional characteristic. It was found that monocytes had decreased values of adhesion as compared with controls (12.0 ± 3.2 vs. 42.7 ± 1.16 Units, $P < 0.05$) and of spreading as compared with controls (14.0 ± 3.0 vs. 30.3 ± 3.1 Units, $P < 0.05$). Examination of C3 receptor expression in an EAC rosette formation test revealed a strong suppression of these receptors. The relative number of EAC rosette forming monocytes declined to 23.4 ± 2.4% compared with 34.1 ± 2.3% in controls. At the same time, the amount of lysosome luminescence in the monocyte cytoplasm was increased by 1.3 - fold ($P < 0.05$).

2. After 10-12 years of service at the antimony

Figure 5: Correlational pleiad of immune indices in workers of the immunodeficiency risk group 2.



plant, the second variant of immunodeficiency develops. Deficits in T cell immunity become permanent. The absolute and relative count of T lymphocytes is decreased as compared with controls ($P < 0.05$). There is an abnormal change in T cell subpopulations as seen from increased theophylline sensitive cells and decreased theophylline resistant cells (18.0 ± 2.0% and 29.03 ± 3.6%, respectively), the background levels of which do not become restored in subsequent years. Tests for T helper and suppressor cell subpopulations using monoclonal antibodies confirmed these results. The count of B lymphocytes is normal and even somewhat reduced. In 78.0% of workers IgE content was elevated 2-3 times above the normal level. The absolute and relative count of EAC rosette forming monocytes, the abilities of monocytes to adhere and spread were reduced. Lysosome luminescence in monocyte cytoplasm was increased (total lysosome luminescence index: 517 ± 3.88 Units), but the amount of diformazan positive cells was significantly decreased in a nitroblue tetrazolium test ($P<0.05$). These changes in immunity induced by antimony exposure indicate that the immune system undergoes readjustment with the formation of a new norm of functioning on a qualitatively lower level of adaptation signifying the beginning of pathological changes.

3. Finally, workers with 12-15 and more years of service develop a third variant of immunodeficiency. It is characterized by reductions in the amount and func-

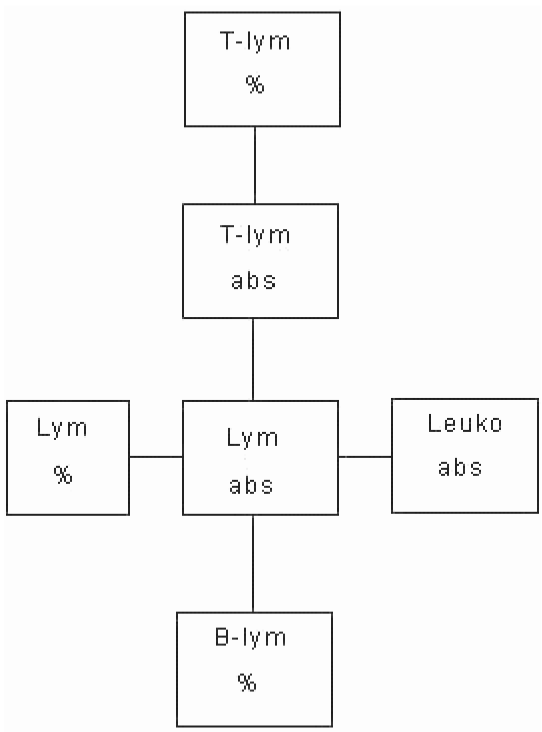


Figure 6: The core of persistent associations between the immune indices in the correlational pleiad.

tional activity of all immunocompetent cells. Numbers and percentages of T and B lymphocytes are decreased ($P < 0.05$). The distinguishing feature is a low activity of T lymphocytes as compared with controls (stimulation index: 17.0 ± 0.9 vs. 21.0 ± 1.4, $P < 0.05$). There is an imbalance of immunoglobulins consisting in decreased IgM and IgA levels ($P < 0.06$) and increased IgG levels ($P < 0.05$). Phagocytosis is decreased ($P < 0.05$). The number of peripheral monocytes is reduced. Their functions such as Fc and C3 receptor expression, adherence, spreading, nitroblue tetrazolium test values are reduced by 1.8, 4.2, 1.8, 3.7 fold respectively. All this eventually leads to irreversible chronification of infections and often to occupational disease. It should be pointed out that a long-term exposure of workers to occupational antimony production factors results in decreased resistance to infection.

In summary, the results of this immunoepidemiological study show that mean values of immune parameters are somewhat higher in residents of the antimony biogeochemical region than in the population of the control region with practically no antimony exposure. The long-term exposure to low antimony levels causes nonspecific activation of some immune parameters and the continual exposure to high levels of antimony leads to the development of immunodeficiency. 23.49% of workers studied made up a group of primary risk for immunodeficiency and 3.62%, a group of increased risk for immunodeficiency which was manifested by a decrease in T and B lymphocyte levels, depression of phagocytosis, an immunoglobulin imbalance with decreased IgA and IgM and increased IgG levels. The number and functions of monocytes were also reduced. Marked immune deficiencies were found in workers of main professional groups (founders, operatives) as compared with workers from auxiliary departments. There was a direct relationship of the extent of immune abnormality to the length of service at the antimony plant. Apparently healty workers experience the period of "adaptation storms" due to high antimony exposure. In workers of primary and increased risk groups the immune system reaches a new stabilization on a lower level of adaptation with development of persistent immunodeficiency and chronic infections.

REFERENCES

1. Bluger LF, HM Veksler and Novitsky : Clinical immunology of intestinal infections. Riga, Latvia, p 214, 1980.

2. Petrov RV, RM Khaitov, IV Oradovskaya, OF Yeremina and MZ Saidov : Assessment of human immune status in large populations. Guidelines for research and health care workers. Immunologia, 6:51-62, 1992.

3. Sokolov VV and EI Rendel : Morphofunctional studies of monocytes as a method for evaluating the state of mononuclear phagocytes. Methodological guidelines. Moscow, p 13, 1983.

4. Vinogradov GI : Theoretical and practical aspects of the norm-setting for environmental factors which uses an immunologically related hazard criterion. Gigiiena i Sanitariia, 4:4-6, 1984.

5. Freudlin IS et al. : Methods of analysing phagocytosing cells in the assessment of human immune status. Leningrad, p 37, 1986.

6. Frankenburg S : A simplified microtechnique for measuring human lymphocyte proliferation after stimulation with mitogen and specific antigen (JIMO4863). J Immunol Meth, 112:177-182, 1988.

7. Schütt H : Blastic transformation reaction of lymphocytes. In: Immunologic Methods, Meditsina, Moscow, pp 294-302, 1987.8. Park BH, SM Fikrig and EM Smithwick : Infection and nitrobluetetrazolium reduction by neutrophils: a diagnostic aid. Lancet, 11:532-534, 1968.

9. Cytochemical studies of leukocytes. Age-related variation in cyctochemical indices. Ed by VB Letsky, Leningrad, pp 7-9, 1973.

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