Ecological and biological aspects of the impact of industrial pollution on cellular and humoral immunity of residents of the Arctic latitudes (on the example of the Murmansk region and the Republic of Karelia)

IOP Conf. Series: Earth and Environmental Science **302** (2019) 012082 doi:10.1088/1755-1315/302/1/012082

Ecological and biological aspects of the impact of industrial pollution on cellular and humoral immunity of residents of the Arctic latitudes (on the example of the Murmansk region and the Republic of Karelia)

A Trotsenko, N Filatov, L Milyakova and M Pankratova

Murmansk State Arctic University, Murmansk, Russia

trotcenko2007@yandex.ru

Abstract. The article presents the results of years of research aimed at investigating the influence of various immunotropic environmental factors on the nonspecific immunity of residents of the Murmansk region and the Republic of Karelia. The authors studied 2011 adults (adults) from 2001 to 2016, namely the indicators of nonspecific immunity - cell and humoral immunity of a representative sample: buccal test, skin bactericidal activity, cytochemical study (glycogen, alkaline phosphatase) of peripheral blood and white cells blood count. The subjects' gender, age, length of residence in northern latitudes, the presence of chronic diseases, etc. were analyzed in detail. It describes the statistical data of target regions, level of industrial pollution, and features of climatic conditions. The studied indicators of nonspecific resistance of the residents of the Murmansk region, on average, are within the normal range, in contrast to similar indicators in the inhabitants of Karelia. An analysis of the research has shown that blood parameters determine compensatory-adaptive processes in combination with indicators of nonspecific immunity when a person adapts to adverse conditions of industrial cities and climatic factors. The emergence of such dependencies indicates the activation of metabolism and increase the work of reserve immune systems to maintain life safety. The following blood counts reacted to the regional and industrial factors: glycogen in lymphocytes and neutrophils, basophils, monocytes, leukocytes, segmented neutrophils. The methods of research of nonspecific resistance of the organism can be recommended as one of the criteria for the influence of environmental factors on the state of the human body as a whole.

1.Introduction

The negative effect of industrial pollution on human immunity seems natural and has been confirmed by numerous studies. The relationship of blood values with the immune system and the environment is obvious. However, blood levels adapt faster and more actively to climatic factors than to industrial ones.

Nonspecific resistance of the organism (constitutional immunity) is determined and depends exclusively on the bactericidal properties of the skin and mucous membranes, on the degree of phagocytic activity (prompt and accurate response of macrophages and polymorphonuclear leukocytes to foreign body components) and on timely participation in non-specific immune protection of humoral factors (system of various antimicrobial proteins).

2.Formulation of a problem

Considering the intensive industrial development of modern society, the progress of the mining industry in Russia and the development of the Arctic region, constant monitoring of the state and activity of all components of the human immune system living in extreme climatic conditions and the permanent impact of the industries on the organism becomes topical and relevant.

The component blood composition of a healthy person is relatively constant due to the clear coordination of the processes of blood formation and destruction. Uniform elements (platelets, erythrocytes, and leukocytes, represented by granulocytes (neutrophilic, eosinophilic and basophilic polymorphonuclear) and agranulocytes (lymphocytes and monocytes)) determine the clinical scores of human peripheral blood [4]. Lymphocytes are another indicator of metabolic processes occurring in the internal organs, the cytochemical analysis of which allows to evaluate the subtle processes of cellular metabolism at the molecular level, and hence the change in the functional state of the whole body.

3.Methods of research

From 2001 to 2016 indices of nonspecific immunity of 2011 adults of the Murmansk region (n = 996) and the Republic of Karelia (n = 1015) were studied: buccal test, skin bactericidal activity, cytochemical study (glycogen, alkaline phosphatase) of peripheral blood and differential blood cell count [4] (venous blood, taken for subjects during the routine examination in hospitals, all necessary documents on the legality of the procedures were signed by the interested parties of the medical institution). The following data were analyzed in detail: sex, age, duration of residence in northern latitudes, the presence of chronic diseases, etc. The anthropogenic impact and climatic conditions of the study areas were assessed according to all available official statistics.

Determination of the bactericidal activity of the skin was determined by the method of Klemparskaya N.N. using the broth culture of Esherichia coli, diluted 5x108 times (in our modification 250 microbial bodies in 1 ml, i.e. 2.5x108). The bactericidal index of the skin of healthy people is 90-100%.

The buccal test was performed by the method of N.N. Belyaeva. The imprint smear of the buccal mucosa was stained by Gram and the microflora was identified under a microscope, evaluating the following values:

- number of streptococci. It is normally around ≈ 100 cells per mm2;
- total microbial count without streptococci (TMC). TMC is normally ≈ 50 cells per mm2;
- ratio of streptococci and TMC (CDB - the coefficient of differentiation of buccal microorganisms). The normal ratio of streptococci to TMC amounts to 2:1.

Cytochemical studies of blood cells were performed by the standard method:
Biochemical indicators - the average cytochemical coefficient (K):
glycogen in neutrophils (normal range: 2.09 – 2.99);
glycogen in lymphocytes (normal range: 0.02 – 0.52);
alkaline phosphatase (normal range: 0.115 – 0.15).
Quantification of leukocytes in 1 l of blood (normal range: 4 – 11 * 109):
The qualitative composition of leukocytes -% of the total number of leukocytes:
monocytes (normal range: 3 - 8);
band neutrophils (normal range: 1 - 6)
segmented neutrophils (normal range: 35 - 70)
lymphocytes (normal range: 20 - 50)
eosinophils (normal range: 1 - 5)
basophils (normal range: 0 - 1)

The validity of the results was evaluated using the Fisher statistical test for independent samples, compared in pairs, with the statistical significance of $p \leq 0.01$, taking into account the variance factor. The group error probability was controlled with Bonferroni correction and the critical level of significance was 0.015. The statistical significance, nature and strength of the relationship between indicators of nonspecific immunity was carried out using the Pearson correlation coefficient (significant correlation $r_{xy} \geq 0.7$ with $p \leq 0.01$).

4.Results

The influence of the industries on the blood formula of the residents of the Republic of Karelia (n = 420 from the total sample of subjects tested residents of the Republic) and the Murmansk region (n = 481 from the total sample of subjects tested in the Murmansk region) was studied. The figure 1 presents the analysis of statistical data on emissions and discharges into the environment of industrial waste in the target regions (average values from 2001–2016) [1, 6].

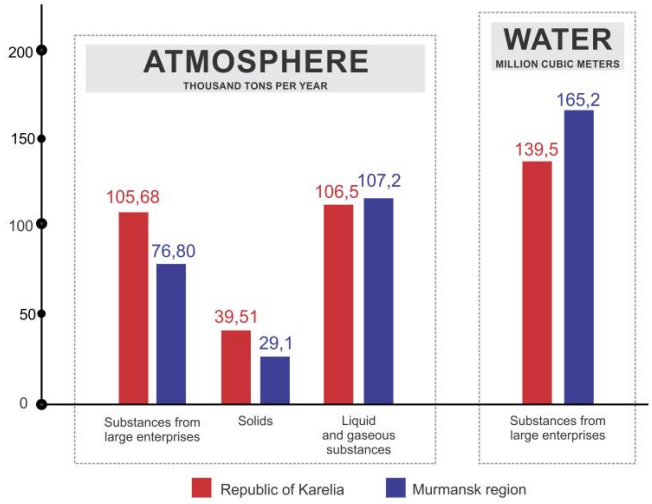

Figure 1. Quantitative averaged data on emissions and discharges of industrial waste in the study regions from 2000 to 2016

It should be noted that the Murmansk region is not an industrial area; moreover, in the spring of 2018, according to the analysis of the “Green Patrol” Board the Murmansk Region took the sixth place in the ranking of the most ecologically safe regions of Russia. Officially, the Murmansk region has a zero (out of three) industrial pollution index. The Republic of Karelia has an industrial pollution index of 2 out of three, given that there are only 24,356 industrial facilities in the Republic, and 742 in the Murmansk Region (as of May 2018) [6]. The difference in industrial pollution of the environment from industrial production is obvious.

Peripheral blood indices are interrelated with the barrier characteristics of nonspecific resistance of the subjects (the main component: bactericidal activity of the skin, the total microbial number of the oral mucosa and the number of streptococci in the oral cavity). The dependence of non-specific resistance of all subjects on sex and presence or absence of an industrial impact was investigated ($n = 2011$; $p \leq 0.01$) [8].

It was revealed that in each territorial region there were no significant sex differences in the studied signs of non-specific immunity. However, when comparing the results of these regions, significant differences are found - women and men living in polar conditions have more adapted and high immune status than the sexes of similar age categories in the Republic of Karelia.

The differences in the sex composition of the subjects from Karelia and the Murmansk region cannot explain the observed regional differences in the immune status. In part, these differences can be explained by climatic features: in the Murmansk region, the climate is milder, namely, the annual difference between the maximum and minimum monthly average temperatures is less. To a large extent, a higher level of immunity among residents of the Murmansk region may be a consequence of a higher standard of living in this region: per capita income and wages of residents of the Murmansk region are one and a half to two times higher than those of Karelia. Undoubtedly, regional differences in the level of immunity are affected by differences in the migratory composition of the population of the two regions. Similar studies confirm our results. However, the influence of the migration factor may simply reflect regional differences in the standard of living [7, 8].

According to the results, the change in blood parameters in all subjects reflects the effect of industrial pollution (Table 1).

Table 1. Differential blood cell count and biochemical indices of peripheral blood relative to the norm range (N) of the inhabitants in the target regions

Peripheral Blood Indicators	**Republic of Karelia**		**Murmansk region**	
	Non-industrial areas	Industrial areas	Non-industrial areas	Industrial areas
Differential blood cell count				
Monocytes	N	N	N	N
Band neutrophils	N	N	N	N
Segmented neutrophils	N	N	N	N
Lymphocytes	N	N	N	N
Eosinophils	N	N	N	N
Basophils	N	> N	N	> N
White blood cells	N	N	N	N
Biochemical indices				
Glycogen in neutrophils	N	N	N	N
Glycogen in lymphocytes	> N	> N	N	> N
Alkaline phosphatase	N	N	N	N

It is obvious that all blood components are interrelated and constitute the second main component, which can be compared with the first one by nonspecific resistance. It is known that all blood cells are grouped by their function [4]. This is confirmed in the present work, namely - three functional blood groups were distinguished among the criteria of the peripheral blood of all subjects by the components: 1 - monocytes, lymphocytes, band neutrophils, segmented neutrophils - blood components responsible for the general immune response of a person; 2 - eosinophils, basophils, alkaline phosphatase - blood components, reflecting the autoimmune conditions of the body and hypersensitivity to various factors; 3 - glycogen in neutrophils, glycogen in lymphocytes, leukocytes in general - blood components, providing energy immune costs.

The presence of 95 percent confidence limits allows an approximate estimate of the statistical significance of differences between averages — if the boundaries do not overlap, the averages differ at a significance level of less than 5%.

Neutrophils are the most variable group of leukocyte cells. Their main function is the fight against foreign agents (toxins and bacteria) and the release of proteolytic enzymes for resorption of necrotic tissue and wound healing. The stab neutrophils are younger, immature forms, and the segmented ones, on the contrary, are more mature.

The number of stab neutrophils in the peripheral blood of all subjects is within the normal range (N = 1 – 6 %).

Results on segmented neutrophils are somewhat different. Even though all indicators are within the normal range (N = 35–70%), the average values of segmented neutrophils in the residents of Karelia in both industrial and non-industrial regions are statistically different. Also, there is statistically significant difference by regional factor in non-industrial regions.

Lymphocytes produce a response when a foreign antigen is introduced into the body. There is no statistical difference between the regional and industrial factors in the content of lymphocytes in the blood of residents of industrial and non-industrial regions and is within the normal range. (N = 20 - 50 %).

Basophils are multifunctional: they support the movement of blood in the capillaries and their restoration, provide for the migration of other leukocytes into the intercellular substance, and participate in delayed-type allergic and inflammatory reactions in different tissues.

The number of basophils in the blood of all subjects from non-industrial regions is within the normal range (N = up to 1%), and among residents of industrial cities it is slightly higher than the normal range. At the same time, the differences between these indicators for the residents of two subjects are statistically insignificant.

Obviously, this indicator reflects the hypersensitivity of the organism to environmental pollutants, considering the industrial pollution. These results also show a tendency to immune tension, increased «readiness» of the body to give allergic reaction. Allergic diseases among the population of the Northern regions of the Russian Federation are relatively rare, due to the low population density and low distribution of allergens in the environment. They were found out only in 0.3% of the indigenous population of the Murmansk region, and in the Republic of Karelia - 8.3% [3].

Alkaline phosphatase is involved in the metabolism of phosphorus and is responsible for autoimmune states, hypersensitivity to various factors. The average values of alkaline phosphatase in most subjects are within the normal range (N = K = 0.120 - 0.150), except for residents of non-industrial cities of Karelia. There is also a noticeable statistical difference in alkaline phosphatase indicators among residents of non-industrial cities of Karelia and the Murmansk region and a significant difference in the phosphatase indicators within the region itself, Karelia. It is possible that blood biochemical parameters are sensitive to industrial pollution.

Glycogen in lymphocytes and neutrophils is localized in the cytoplasm and plays a leading role in energy metabolism. All subjects (N = K = 2.09 - 2.99) have glycogen in neutrophils within the normal range. Regional variability of the index, i.e. the difference between the average values of glycogen is significant: in industrial cities of Karelia, the values are lower than in any city of the Murmansk region, and the average values for the residents of the Murmansk region are higher.

However, the situation with glycogen levels in lymphocytes is different. All values in all regions are above the norm (N = K = 0.02 - 0.52), but there are no statistically significant differences within the groups by region and industrial pollution.

It can be assumed that the energy component of blood cells creates a "buffer" and the body's readiness for metabolic processes that maintain homeostasis, therefore the glycogen in lymphocytes is increased. Such changes in metabolic parameters indicate the involvement of immune cells (lymphocytes) in the processes of antigen recognition (for example, a pollutant) and the formation of a compensatory nonspecific immune response in general.

On the other hand, an increase in glycogen in blood cells may indicate a deficiency in serum glucose due to its high consumption in other compensatory reactions [5]. This statement requires further research.

Monocytes are macrophages, which, along with neutrophils, are the main phagocytes. All indicators are within the normal range (N = 3 - 8%). There is no statistical difference in the industrial pollution in each region for monocytes. However, there is a significant regional difference. Probably, the climatic features are reflected in the phagocytic function of the blood, i.e. non-specific blood immunity.

Eosinophils, like basophils, are responsible for the body's hypersensitivity to various factors, and are involved in inflammatory and allergic reactions. All indicators in our studies are within the normal range (N = 1 - 5%) and there is no statistically significant difference for either regional or industrial factors.

Leukocytes are white blood cells that include the above listed leukocytal cells. They are divided into three main groups: granulocytes, monocytes and lymphocytes. In our research this indicator in all regions of the study is within the normal range (N = 4 - 11 * 109/l). However, inside the district, in Karelia, there is a significant increase in leukocytes in industrial settlements. It is assumed that the overall immune status of residents of industrial cities of Karelia shows a tendency to be more willing to respond to potentially possible other factors than residents of non-industrial settlements of the Republic.

And the residents of the Murmansk region do not have the statistical differences of these factors and have a high immune status on leukocytes.

As mentioned above, the indicators of peripheral blood always reflect changes in the homeostasis of the body due to the impact of various factors. Table 2 shows which blood indicators, despite being within the normal range, turned out to be more sensitive to some factors.

Table 2. Sensitivity of blood parameters to the studied factors

Regional factor	**Industrial factor**
Segmented neutrophils	Segmented neutrophils
Basophils	Basophils
Alkaline phosphatase	Glycogen in neutrophils
Glycogen in lymphocytes	Glycogen in lymphocytes
Monocytes	White blood cells

Since all the subjects were relatively healthy (according to their health passport), it can be assumed that such a reaction of blood cells to an industrial factor just confirms the relative well-being of the immune system of residents in the study regions. Lymphocytes are from the first functional group, that is, they undoubtedly reflect the readiness of the entire immune system to react to the extreme impact of a factor; basophils are from the second functional group, i.e. demonstrate hypersensitivity of the body and allergic readiness; glycogen in lymphocytes is from the third functional group, i.e. reflect energy readiness when exposed to an adverse factor. We also notice the tendency to enhance phagocytic nonspecific immune response among residents in different regions of the study. There are similar data on the change in adolescents with non-specific resistance. For example, in the works of N.V. Dorshakova, a decrease in nonspecific immunity, namely, lysozyme of saliva, lymphocytes and phagocytic activity of peripheral blood neutrophils in adolescents from industrial areas of the Republic of Karelia (Segezhsky district, Pitkyaranta) [2], is described. This fact is associated with chronic fluoride intoxication from industrial enterprises of these cities, which, in turn, causes violations of the nonspecific immunity of the oral cavity in the form of inhibition of the bacteriostatic properties of the mucous membrane. The norms of laboratory results, due to their universality, do not always consider the diversity and peculiarities of the human environment.

5.Conclusions

Blood parameters determine compensatory-adaptive processes in combination with indices of nonspecific immunity when a person adapts to adverse conditions of industrial cities and climatic factors. The emergence of such dependencies indicates the activation of metabolism and the adaptive capabilities of humoral and cellular immunity in humans. Adaptive mechanisms of cellular immunity were revealed among the following blood parameters: glycogen in lymphocytes and neutrophils, basophils, monocytes, leukocytes, segmented neutrophils. These methods of research of nonspecific resistance of the organism can be recommended as one of the criteria for determining the influence of environmental factors on the state of the human body.

References

[1] Report on the state and protection of the environment of the Murmansk region in 2016 2017 180
[2] Dorshakova N V *et al* 2002 The state of the immune system of the child population living in different environmental conditions of the Republic of Karelia *Vestnik RUDN* **2** 28 -33
[3] Zubatkina O V 2002 Ecological - physiological features of a person’s metabolic status and its correction in the European North of Russia (Arhangelsk, Pomor. gos. un-t im. M. V. Lomonosova) 30
[4] Kozinets G I 1997 The study of the blood system in clinical practice (Moskva,Triada – X) 480
[5] Nazarenko G I and Kishkun A A 2006 Clinical evaluation of laboratory results (Moskva,Meditsina) 541
[6] Rossstat 2016 Regions of Russia. Main characteristics of the constituent entities of the Russian Federation Available from: http://www.gks.ru/doc_2016/region/subject.zip [Accessed 20th March 2019]
[7] Trotsenko A A 2011The influence of the environment on the nonspecific immunity of the inhabitants of the Republic of Karelia and the Murmansk region (Moskva, MGU im. M.V. Lomonosova) 102
[8] Trotsenko A A *et al* 2010 The impact of demographic and climatic factors on the nonspecific immunity of residents of the Republic of Karelia and the Murmansk region *Narodonaselenie* **1** 113 - 119

[9] Braff M H *et a*l 2005 Cutaneous defense mechanisms by antimicrobial peptides *J. Invest Dermatol* **125** 9–13
[10] Bascomb R *et al* 1996 Health Effects of Outdoor Air Pollution *Am J. Respir Crit. Care Med* **153** 477-498
[11] Maximov A L 1993 Cold adaptation and related phenomena in the Indigenous and Immigrant populations in the Russian North-East *Proc. IX Int. Congress on Circumpolar Health (Reykjavik, Iceland, June 20-25, 1993)* 19
[12] McDade T W 2003 Life history theory and the immune system: steps toward a human ecological immunology *Am. J. Phys. Anthropol* **37** 100-125
[13] McDade T W 2005 The Ecologies of Human ImmuneFunction *Am. J. Phys. Anthropol* **34** 495-521
[14] Hamady M and Knight R 2009 Microbial community profiling for human microbiome projects: Tools, techniques, and challenges *Genome Research* **19** 1141 - 1152
[15] Wintrobe M M *et al* 1993 *Wintrobe's clinical hematology* (Philadelphia) 2606