A.V. Samodova, L.K. Dobrodeeva

THE ROLE OF DEFICIENCY OF THE CONTENT OF CIRCULATING LEUKOCYTES IN THE CONSERVATION OF IMMUNE STATUS IN PEOPLE IN LIFE CONDITIONS ON THE SPHITZBERGEN ARCHIPELAGE

ABSTRACT

The **purpose** of the study is to establish the role of the deficiency in the content of circulating leukocytes in preserving the immune status of people in the conditions of life on the Svalbard archipelago. We surveyed 74 able-bodied residents of the village of Barentsburg Arch. Svalbard, 45 women and 29 men, aged from 20 to 60 years and 77 healthy people at the time of the survey, living in Arkhangelsk, 64 of them women and 13 men, aged 21 to 55 years. The hemogram, neutrogram, monocytogram and lymphocytogram were studied in blood smears stained according to the Romanovsky-Giemsa method, neutrophil phagocytic activity, lymphocyte phenotypes (CD3 +, CD4 +, CD8 +, CD10 +, CD19 +, CD23 +, CD71 +, CD95 +) by the method of indirect immunopsypoxy. The content of cytokines IL-1β, TNF-α, IL-6, irisin, endothelin-1 and Nt-pro-BNP was determined by ELISA. The concentration of the CEC - by the method of precipitation using PEG-6000. It has been established that neutropenia (32.43%), monocytopenia (28.38%) and lymphopenia (18.92%) are recorded in the working-age population living in the Spitsbergen archipelago. The decrease in the content of circulating neutrophils and monocytes is associated with a sharp increase in the activity and intensity of phagocytosis. On the background of neutropenia and monopenia, there is a deficiency in the content of mature T-lymphocytes (91.89%), activated T-cells with a receptor for transferrin and T-helper cells (44.59%). Against the background of T-cell deficiency, the levels of irisin (45%), Nt-pro-BNP (20%) and endothelin-1 (10%) are increased. Signs of compensatory proliferation of myeloid cells and lymphocytes are not installed. The decrease in the content of neutrophils occurs due to functionally active cells, low concentrations of monocytes, mainly promonocytes, lymphocytes - mostly of small forms, which constitute the majority of recycle cells. It has been established that the risk of T-helper immunodeficiency is very high. An increase in the activity of phagocytes does not fully compensate for the deficiency of their content in the blood, since elevated concentrations of the CEC are very often recorded at 55.41-100%.

Keywords: monocytes, mature T-lymphocytes, T-helpers, activated T-lymphocytes, neutrophil phagocytosis, irisin, brain natriuretic peptide (Nt-pro-BNP), endothelin-1.

Introduction. The influence of adverse climatic factors on the human body is associated with pronounced fluctuations in the functional activity of various systems with the expansion of the limits of fluctuations of almost all physiological parameters, reduction of the reserve capacity to regulate homeostasis. This concerns, first of all, the neuro-immuno-endocrine regulation of the functions of the cardiovascular system. The earliest reactions are from the side of catecholamines and short peptides, which alter the hemodynamics, frequency and strength of heart contractions. Thus, the short peptide endothelin-1 has a powerful vasoconstrictor effect [16]. The brain natriuretic peptide (Nt-pro–BNP) ensures the preservation of intracellular pressure by keeping sodium in the cell against the density gradient [10]. Irisin induces the activation of thermogenin in the cells of brown adipose tissue [14]. One of the pathogenetic factors of cardiovascular crises is the systemic effect of proinflammatory cytokines. Pro-inflammatory cytokines are secreted by any cell in response to a negative factor that threatens the integrity and functional activity of the cell. The physiological effect of cytokines is mainly local, but with an increase in their concentration in the blood, systemic damage may develop. Damage to endothelium cells during an inflammatory reaction is accompanied by their retraction and the formation of gaps [8]. The instability of the immune system during a long stay in the polar region is accompanied by the formation of environmentally dependent immunodeficiencies. It is known that the T-cell homeostasis system is very stable and capable of self-preservation even with the elimination of 15% of the cells [11]. A secondary violation of the T-cell repertoire, up to immunodeficiency, usually develops through a violation of T-cell clonal equilibrium due to prolonged or extreme predominance of activation. The aim of the work is to establish the role of the deficiency in the content of circulating leukocytes in preserving the immune status of people in the conditions of life on the Svalbard archipelago.

Material and methods of research. We surveyed 74 able-bodied residents of the village of Barentsburg Arch. Svalbard, 45 women and 29 men, aged from 20 to 60 years, during the polar day (July-August 2017) and 77 healthy people at the time of the survey are people living in Arkhangelsk, 64 of them are women and 13 are men aged 21 to 55 years. All studies were carried out with the consent of the volunteers and in accordance with the requirements of the Helsinki Declaration of the World Medical Association on the ethical principles of medical research (2000). The complex of immunological research included the study of the number and ratio of cells of hemogram, neutrogram, monocytogram, lymphocytogram, phagocytic activity of neutrophilic peripheral blood leukocytes in blood smears stained by the Romanovsky-Giemsa method. The selection of mononuclear cells from peripheral blood was performed according to the method of A. Boum [6]. Phagocytic activity of neutrophils was studied after incubation of 100 μl of citrated blood and an equal amount of reagent with Reactlex production latex at 37 ° C for 30 minutes. The phenotypes of peripheral blood lymphocytes (CD3 +, CD4 +, CD8 +, CD10 +, CD19 +, CD23 +, CD71 +, CD95 +) were studied by indirect immunoperoxidase reaction (Sorbent reagents, Moscow). The content of cytokines IL-1β, TNF-α, IL-6, irisin, endothelin-1 and brain natriuretic peptide (Nt-pro-BNP) was determined by enzyme immunoassay in the serum on an automated immuno-enzyme analyzer "Bio-RAD" (Germany). The concentration of circulating immune complexes was determined by the standard precipitation method using 3.5%; four%; 7.5% PEG-6000. Mathematical and statistical analysis of the research results was carried out on a IBM / AT-Pentium 4 computer using the Microsoft Excel 2010 (USA) and Statistica 7.0 application software (StatSoft, USA).

Results and discussion. The inhabitants of the Spitsbergen archipelago have a lower total leukocyte count in pe-

ripheral venous blood (5.07±0.18 and 5.80±0.19X10^9 cells/l; p <0.01) due to neutrophilic granulocytes (2.48±0.11 and 2,96±0,14X10^9 cells/l; p<0.05) mainly with 2 and 3 segments of the nucleus (respectively, 0.62 ± 0.03 and 0.85±0.05X10^9 cells/l; p <0,01 and 1.02±0.05 and 1.24 ± 0.06X10^9 cells/l (p <0.01); neutropenia is registered in 32.43% of the patients (24 people). The deficit of active phagocytes is set at 14.71% and only in 4.48% of cases coincides with neutropenia. In individuals with neutropenia, a high level of phagocytic protection was recorded: 71.33 ± 4.66% with an intensity of phagocytosis - 9.67±1.74 pcs. The residents of the village. Barentsburg increased neutrophil phagocytic activity level was detected in 30.88% (29 people) and averaged 91.0 ± 1.38% with phagocytosis intensity - 17.9 ± 1.75 pcs. It is known that adverse environmental factors in the early stages increase the activity of phagocytes, but further, reducing the reserve capacity, reduce the activity of phagocytosis and its intensity [1]. In the structure of the hemogram in the examined individuals of the Arctic settlement, the content of monocytes is lower (0.22±0.02 and 0.37±0.03X10^9 cells/l; p<0.001) mainly due to promonocytes (0.03±0.002 and 0.16±0.02X10^9 cells/l; p <0.01) and lymphocytes (2.19±0.09 and 2.33 ± 0.11X10^9 cells /l; p <0.05) of mostly small forms (1.20±0.05 and 1.42± 0.08X10^9 cells/l; p <0.01), which are known not only to circulate intensively, but are also capable of recycling [7]. Monocytopenia and lymphopenia were detected in 28.38 and 18.92% of the examined individuals, respectively. It is known that a decrease in the content of leukocytes is the main signal for admission of the corresponding cells from the depot to the circulation [7]. So, the main neutrophil depot is the lung capillary network. The tissue pool of monocytes is 3.5 times higher than the content of tissue neutrophils [13], so the migration processes of monocytes from the blood may seem less intense and appear less pronounced. There is an assumption that monocytes are recyclable [7]. Practically in all examined persons (91.89%), there was a deficiency in the content of mature T-lymphocytes (CD3 +), the average level of which was (0.61±0.03)X10^9 cells/l. The low level of activated T-cells with transferrin receptor (CD71 +) (0.32±0.02)X10^9 cells/l is noteworthy, in 87.83% of cases there was a deficit of these cells, which makes it possible to judge the intensity of anaerobic metabolism in people living in the arctic territory. 10.81% of the inhabitants of the archipelago increased the content of cytotoxic lymphocytes (CD8 +), the average level of which was (0.38±0.02)X10^9 cells / l. In 44.59% of the examined, there was a deficiency in the content of T-helper cells, the average content of which was (0.48±0.03)X10^9 cells/l). A low content of immunocompetent cells labeled for programmed death by the Fas receptor was established, which corresponds to a low level of lymphoproliferation activity. Thus, the content of lymphocytes with the CD95 receptor was (0.34±0.02)X10^9 cells/l and in 87.83% of cases the level of these cells was lower than 0.6X10^9 cells/l. Against the background of insufficient content of mature T cells in the inhabitants of the archipelago, only 13.51% of cases showed signs of increased lymphocyte proliferative activity with an increase in the content of lymphocytes with CD10 receptor in the peripheral blood (0.44±0.03)X10^9 cells/l, as well as elevated levels of B lymphocytes with the CD19 receptor and the Fc receptor for IgE (0.43 ± 0.03) and (0.38 ± 0.03)X10^9 cells/l, registered respectively in 14.86 and 20.27% of cases. Against the background of T-cell deficiency, residents of Spitsbergen most frequently recorded elevated concentrations of short peptides: irisin in 45% of the subjects, brain natriuretic peptide (Nt-pro-BNP) and endothelin-1, respectively, in 20 and 10% of cases. The content of Nt-pro-BNP, endothelin-1 and irisin among residents of the Arctic village, as well as among residents of the city of Arkhangelsk, is in a very wide range of fluctuations (respectively, 0.56-219.72 and 1.713-267.87 fmol / ml; 0.05-3.84 and 0.073 - 7.018 fmol / ml; 0.001-14.59 and 0.605 - 24.328 μg / ml). The most dramatic differences are very often established with respect to iris; in other words, the problem of heat production is the most important. In the background are the problems of preserving the osmotic pressure of the cell by preserving sodium against the density gradient on the part of the brain natriuretic peptide and on the latter the dysregulation of vasoconstriction with endothelin-1. It is known that the content of irisin is interconnected with the concentrations of the main pro-inflammatory cytokines - TNF-α and IL-6 [12]. Indeed, individuals living in Spitsbergen archipelago have a higher concentration of pro-inflammatory TNF-α (14.05±0.70 and 2.85±0.37 pg / ml, p <0.001) unchanged on the side of IL-1β (11,24±3.22 and 9.63±0.54 pg / ml) and IL-6 (5.59±0.32 and 5.80±0.52 pg/ml). Orientation toward inflammatory cytokines suggests a higher level of reactive oxygen metabolites, a sharp increase in phagocytosis. TNF-α plays an extremely important role in the first moments of the inflammatory reaction, because it activates the endothelium and promotes the expression of adhesive molecules, which leads to the adhesion of granulocytes to the inner surface of the vessel. Under the influence of TNF-α, the transendothelial migration of leukocytes occurs in the tissue [15]. On myocardiocytes there are specific receptors for iris. Through these receptors, irisin is capable of enhancing metabolic processes, increasing mitochondrial biogenesis and increasing oxygen consumption and energy expenditure in myocardial cells, which is accompanied by inhibition of cell proliferation, but also contributes to their differentiation [9]. To prevent the loss of the extracellular pool and maintain the hydrodynamic pressure of the cells, the influence of the brain natriuretic peptide is activated [2]. At the same time, an increased level of Nt-pro-BNP is a risk of hemodynamic disturbances with possible signs of myocardial overload, left ventricular hypertrophy and renin-angiotensin system insufficiency, which provides the level of tissue oxidation [17]. Inhibition of lymphoproliferation and differentiation of lymphocytes with an increase in the content of brain natriuretic peptide in the blood is associated with a deficiency of IL-2 and is due to an increase in the concentration of IL-10. In the blood of the inhabitants of arch. Svalbard recorded elevated levels of circulating immune complexes. Thus, the average content of the CEC IgA is 3.9±0.23 g/l, in 55.41% of cases elevated levels are noted. In 81.08% of the examined individuals, elevated concentrations of IgM CIC were detected with an average content of 6. ±0.40 g/l. Anomalous CIC IgG values were established for all villagers, exceeding the upper limit of the norm by a factor of 13 - 45.3±2.03 g/l. It is known that long-term circulation in the blood of the CEC contributes to the formation of their deposits in the tissues, on the basement membrane of small vessels and capillaries and leads to disruption of microcirculation, blockage of vessels, damage and necrosis of tissues [4]. This is facilitated by violations of blood flow, triggering the adhesion and aggregation of platelets, the release of vasoactive amines and an increase in vascular permeability. Thus, 56.76% of the examined individuals recorded platelet aggregation and their lysis. The CEC interacts with cells that have membrane FcIg receptors for the components of the complement system, which enhances platelet aggregation with the involvement of collagen and fibrin in this process. Abnormally high levels of the CEC can disrupt microcirculation, accompanied by impaired blood supply, changes in migration, exudation and

transudation [3]. **Conclusion**. Thus, neutropenia (32.43%), monocytopenia (28.38%) and lymphopenia (18.92%) are recorded in the working-age population living in climatic and geographical conditions of the Spitsbergen archipelago. The decrease in the content of neutrophils occurs due to functionally active cells, low concentrations of monocytes, mainly promonocytes, lymphocytes - mostly small forms, which, according to the literature, constitute the majority of recycled cells. Small lymphocytes that retain information of antigens and which are predominantly T-cells of the reserve pool, are capable of being recycled, and are capable of further blast transformation and differentiation [5]. The decrease in the content of circulating neutrophils and monocytes is associated with a sharp increase in the activity and intensity of phagocytosis. An increase in phagocytic activity is likely to compensate for a decrease in the concentration of phagocytes in the blood and, possibly, is a reflection of the activity of the reactions of the microcirculatory bed. Against the background of neutropenia and monopenia, there is most often a deficit in the content of mature T-lymphocytes (91.89%), activated T-cells with a receptor for transferrin and T-helper cells (44.59%), i.e. The risk of T helper immunodeficiency is very high. Against the background of T-cell deficiency, the levels of irisin are increased (45%), which indicates the need to increase heat production. Signs of compensatory proliferation of myeloid cells and lymphocytes have not been established: the content of stab neutrophils, promonocytes, as well as large lymphocytes, CD10 + and CD95 + is not increased. The increase in the activity of phagocytes, in all likelihood, does not fully compensate for the deficiency of their content in the blood, since elevated concentrations of CIC are recorded very often (CIC IgA 55.41%, IgM 81.08% and IgG 100%). The voltage of hemodynamic regulation reactions is not so rare; elevated concentrations of Nt-pro-BNP and endothelin-1 were recorded, respectively, in 20 and 10% of the examined individuals.

Acknowledgment

The authors are sincerely grateful to the Doctor of Biological Sciences, the Chief Researcher of the Research Center for Medical and Biological Problems of Human Adaptation in the Arctic of the Kola Scientific Center of the Russian Academy of Sciences Belisheva Natalia Konstantinovna for organizing an expedition to Spitsbergen archipelago and working in expeditionary conditions, as well as great methodological assistance.

Funding

The work was performed in the framework of the program of fundamental scientific research on the laboratory of regulatory mechanisms of immunity of the Institute of Physiology of Natural Adaptations "The role of the extracellular pool of adhesion molecules and short peptides in the formation and outcome of human adaptive responses to changes in the light mode" (No. AAAA-A17-117033010123-0).

REFERENCES

1. Беседин А.В., Калуцкий В.П. Особенности функциональной активности фагоцитов при воздействии магнитных полей различного происхождения. *Вестник уральской академической науки. Тематический выпуск по аллергологии и иммунологии.* 2009; №2/1: 20-21. [Besedin AV, Kalutsky VP. Features of the functional activity of phagocytes when exposed to magnetic fields of various origin *Vestnik ural'skoj akademicheskoj nauki. Tematicheskij vypusk po allergologii i immunologii.* 2009; 2 (1): 20-21. (In Russ.).]

2. Добродеева Л.К., Самодова А.В., Карякина О.Е. Взаимосвязь уровней содержания мозгового натрийуретического пептида в крови и активности иммунных реакций у людей. *Физиология человека*. 2016; 42 (6): 106–115. [Dobrodeeva LK, Samodova AV, Karjakina O.E. The relationship between the levels of brain natriuretic peptide in the blood and the activity of immune reactions in humans. *Fiziologiya cheloveka*. 2016; 42 (6): 106–115. (In Russ.).] DOI: 10.7868/S0131164616050052.

3. Константинова Н.А. Иммунные комплексы и повреждение тканей. М.: Медицина, 1996: 256. [Konstantinova NA. Immune complexes and tissue damage. M.: Medicine, 1996: 256. (In Russ.).]

4. Парахонский А.П. Циркулирующие иммунные комплексы на поздних стадиях сахарного диабета. *Современные наукоемкие технологии.* 2006; 1:79. [Parahonsky A.P. Circulating immune complexes in the late stages of diabetes mellitus. *Sovremennye naukoemkie tekhnologii*. 2006; 1:79 (In Russ.).].

5. Ambrus C.M., Ambrus I.L. Regulation of the leukocyte level. *Ann. N.Y. Acad. Sci.* 1959; 77: 445-479.

6. Boyum A. Separation of leukocytes from blood and bone marrow. *S. Cand. And Clin. Lab. Invest*. 1968; 21: 97.

7. Buckton K.E., Brown W.M, Smith P.G. Lymphocyte survival in nen treated with X-rays for ankylosing spondylitis. *Nature*. 1967; 214: 470–473.

8. Dalmasso A.P., Goldish D., Benson B.; et al. Interleukin-4 induced up-regulation of endothelial cell claudin-5 through activation of FoxOI: Role in protection from complement-mediated injury. *J. Biol. Chem.* 2014; 289 (2): 838.

9. Emanule E., Minoretti P., Pareja-Galeno H. Serum irisin, precocious myocardial infarction, and healthy exceptional longevity. *Am. J. Med.* 2014; 127 (9): 888-890.

10. Hall C. Essential biochemistry and physiology of NT-pro-BNP. *Eur. J. of. Heart. Fail.* 2004; 3: 257.

11. Mahmoud F.F., Al-Salen K.A., Al-Habi S.A., London D.L Involvement if B cells in pathogenesis of nonarthritic psoriasis in a kuwati population. *FASEB J.* 1997; 11 (3): 118.

12. Mazur-Bialy A.I., Bilski J., Pochec E., Brzozowski T. New insight into the direct anti-inflammatory activity of a myokine irisin against proinflammatory activation of adipocytes. Implication for exercise in obesity. *Journal of Physiology and Pharmacology*. 2017; 68 (2): 243-251.

13. *Meuret G., Hoffman G. Monocyte kinetic studies in normal and disease states.* Brit.J. Hematol. 1973; 24: 275–279.

14. Teufel A. Frcp1 and Frcp 2, two novel fibronectin type III repeat containing genes. *Gene*. 2002; 297 (1/2): 79–83.

15. The Cytokine. Handbook. London, 1992.

16. Yanagasawa M. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*. 1988; 332: 411-415.

17. Yao Y., Scheng Z., Li Y. Tissue kallikrein promotes cardiac neovascularization by enhancing endothelial progenitor cell functional capacity. *Hum. Gen. Ther.* 2012; 23 (8): 859.

Authors:

Federal State Budgetary Institution of Science Federal Research Center for Integrated Study of the Arctic named after Academician N.P. Laverov, Russian Academy of Sciences, Institute of the Physiology of Natural Adaptations, Arkhangelsk, Russia:

SAMODOVA ANNA VASILYEVNA, candidate of Biological Sciences, head of the laboratory of regulatory mechanisms of immunity of the Institute of Physiology of Natural Adaptations of the Federal State Budgetary Institution of Science of the Federal Center for Complex Arctic Studies of the Russian Academy of Sciences. acad. N.P. Laverov. E-mail: annapoletaeva2008@yandex.ru.Office phone: (8182) 21-04-58.

DOBRODEEVA LILIYA KONSTANTINOVNA, doctor of Medical Sciences, Professor, Honored Worker of Science, Chief Researcher of the Laboratory of Regulatory Mechanisms of Immunity, Director of the Institute of Physiology of Natural Adaptations of the Federal State Budgetary Institute of Science of the Federal Center for Complex Arctic Studies of the Russian Academy of Sciences. acad. N.P. Laverov, E-mail: dobrodeevalk@mail.ru, Office phone: (8182)652992.