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The Structural Organization of Thymus and Cellular Composition of Blood Under Experimental Exposure to Cold

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ABSTRACT
The immune system is a unique defence mechanism. Due to the well-coordinated work of the entire functional immune system, the body can resist numerous amounts of negative factors. Cold exposure is one of the factors that negatively impact the body. The effect of experimental cold stress on the morphofunctional state of the thymus and the cellular composition of the blood of rats, depending on the time of their exposure, was studied. The effect of cold exposure was studied by placing animals in individual cages in the "Vestfrost" refrigerator (Denmark) at a temperature of 10±2 °C for 1 hour at the same time of the day for 7, 14, 21, and 30 days. Analysis of the research results showed that on the first and late days of the experiment, there is a redistribution of the volume and cellular composition of various structural and functional zones, indicating a decrease in the functional activity of the thymus, an increase in the death of lymphocytes in the process similar to apoptosis, a decrease in mitotic activity and the accumulation of macrophages. At the same time, on the 14th day of the experiment, the development of compensatory-adaptive changes in the thymus in response to cold exposure, manifested in the form of activation of cell division in the cortex and the cortical-medullary zone, is noted. The results of a study of the cellular composition of blood showed that as a result of exposure to cold, a specific immune response is stimulated, as indicated by an increase in the number of lymphocytes in the blood. A decrease in the number of leukocytes indicates suppression of nonspecific cellular immunity in experimental animals.

Keywords: *immune system, thymus, cold exposure, morphometry, peripheral blood, lymphocytes*

1. INTRODUCTION

The immune system is a unique natural defence mechanism that plays a critical role in maintaining the body's homeostasis. Due to the well-coordinated work of the entire functional immune system, the body can withstand many factors that harm the body. The adequacy of the body's response to various damaging factors of exo- and endogenous origin depends on the state of the immune system and its adaptive capabilities, immune system also protects against genetically foreign factors: microorganisms (bacteria, viruses, fungi), foreign molecules, etc. [1–4].

Cold is one of the most common stressors that creates uncomfortable conditions for human existence and affect human health. It is known that the human body responds to the negative impact of various environmental factors by violating the state of the immune system, especially local immunity. Morphologically and functionally damaged organs of the immune system are unable to protect the human body from external or internal threats [5, 6].

In living conditions of the Far North, disturbances in the normal functioning of the immune system lead to the spread of acute and chronic infectious and inflammatory diseases of the respiratory tract, allergic and autoimmune processes, malignant neoplasms of a certain localization among residents [7–9].

The thymus, like the bone marrow, is the central organ of immunogenesis, the state and activity of which largely determines the severity of the defence reactions of the whole organism [10–13]. The morphofunctional state of the thymus and its role in the regulation of lymphopoiesis is of great importance in the implementation of adaptational mechanisms in response to stress caused by cold and is accompanied by a change in the cellular composition of the blood [12, 14].

It is shown that leukocytes play an important role in the implementation of the protective reaction of the body. Since the phagocytic activity of leukocytes is a non-specific cellular immunity, their activity and numbers depend on the impact of any stress factors. Then this is accompanied by changes in immunological reactivity, as well as a decrease in the adaptive capabilities of the body and the development of transient or persistent forms of secondary immune insufficiency [8, 15, 16].

The study of the central immune organs reaction and the mechanics of cellular adaptation of the blood system at low temperatures is an extremely important task given the living conditions in the Republic of Sakha (Yakutia), it is also of great importance for finding ways to increase the body's resistance, prevent and treat immune response disorders, improve the quality of life and life expectancy of people in the North.

This study aims to contemplate the effect of experimental cold-infused stress on the morphofunctional state of the thymus and the cellular composition of the blood of rats, depending on the time of their exposure.

2. MATERIALS AND METHODS

The study used white adult male rats with a bodyweight of 200–300 g (n=40). The animals were divided into 5 groups, 8 rats in each: 1st group – intact animals kept in normal vivarium conditions, 2–5 groups – animals exposed to cold. The effect of cold exposure was studied by placing animals in individual cages in the "Vestfrost" refrigerator (Denmark) at a temperature of 10±2 °C for 1 hour at the same time of the day for 7, 14, 21, and 30 days. The experiment was conducted in a single vivarium, the animals were kept in arbitrary conditions. The animals were removed from the experiment by decapitation after light ether anaesthesia on days 7, 14, 21 and 30.

The protocol of the experimental part of the study at the stages of keeping animals, modelling pathological processes, and removing them from experience, corresponding to the principles of biological ethics set out in the International Recommendations for Conducting Biomedical Research using Animals (1985), the European Convention for the Protection of Vertebrate Animals Used for Experiments or for Other Scientific Purposes (Strasbourg, 1986), order of the Ministry of Health of the USSR No. 755 of 12.08.1977 "On Measures for Further Improvement of Organizational Forms of Work Using Experimental Animals", Order of the Ministry of Health of the Russian Federation No. 267 of 19.06.2003 "On Approval of the Rules of Laboratory Practice".

The rats were decapitated in compliance with the requirements of humanity following Appendix No. 4 "On the procedure for euthanasia (killing) of an animal" to the Rules for carrying out work using experimental animals (appendix to the Order of the Ministry of Health of the USSR No. 755 of 12.08.1977).

Blood samples and serum were obtained during the decapitation of the animal. Haematological studies were carried out immediately after receiving samples on the automated haematological analyzer "AbacusJunior 30", biochemical studies were done on the Mindray BA-88A biochemical analyzer with ready-made HigtTehnology solutions.

The thymuses were fixated with a 10 % buffered formalin solution, the subsequent filling in paraffin was done in a standard way. To carry out a morphological examination, serial sections with a thickness of 5 microns were made, stained with hematoxylin-eosin.

The morphometric study was carried out using the ImageJ software in 25 fields of view with an area of 2500 microns^2 on 5 sections of the central parts of the thymus lobules using oil immersion with an x10 increase in the eyepiece and the x100 increase in the lens. The calculation was performed in the subcapsular, central cortical, cortical-medullary and central cerebral zones of the thymus. The relative areas of the cortical and medullary matter were determined by the method of point counting.

The statistical analysis of the obtained data was carried out using the Statistica 10 software. The reliability of the differences was determined by the Student's t-test, the differences were considered reliable at $p<0.05$

3. RESULTS AND DISCUSSION

Histological examination of the thymus specimen of intact animals revealed a typical lobular structure, clearly distinguishable cortical (57.0±3.87) and cerebral (33.2±1.92) substance. The cortical-medullary index is 1.7±0.12.

The subcapsular zone of the cortical substance contains larger lymphocytes with rounded nuclei, which are mitotically active lymphoblasts. Epithelioretic cells in the subcapsular region have flattened or triangular nuclei, are located outside the cortical substance and form a dense layer.

In the deeper parts of the cortical substance, there is a gradual predominance of mainly small and medium-sized lymphocytes, mitosis is less common. Epithelial reticulocytes are located singly, have a large nucleus, oval or angular shape, and most often with a single nucleolus. The cytoplasm is pale, poorly distinguishable due to a large number of lymphocytes.

In the area of the transition of the cortical substance to the medulla, a large number of vessels are observed, the cellular composition includes lymphocytes, stromal cells, macrophages, plasmocytes of different degrees of maturity.

In the medulla, due to the smaller number of lymphocytes, epithelioretic cells are more observable, they have irregular nuclei with several nucleoli. Among the lymphocytes, medium and large, lighter coloured, mature cells are more common. Macrophages, plasmocytes, and Hassal corpuscles are also detected. The Hassall corpuscles are not numerous, formed by concentrically layered epithelioretic cells with a characteristic structure.

The results of the morphometric analysis of the cellular composition of the morphofunctional zones of the thymus of control animals (Group 1) are shown in Fig. 1-4.

The indicators of the cellular composition of the blood are within normal physiological limits (Table 1). The results are consistent with the data of other authors [17, 18].

Morphometric study of the thymuses of experimental animals that were exposed to cold daily for 7, 14, 21 and 30 days revealed significant changes in all studied groups.

In the 2nd group of animals after a week of exposure to cold, the indicators of the relative areas of the cortical and medulla matter are 48.0±3.53 and 37.4±2.41, respectively, while the cortical-cerebral index significantly decreased to 1.28±0.15.

The analysis of the cellular composition showed a slight decrease in the number of lymphocytes mainly in the area of the cortical substance, but significant differences were noted only in the increase in the number of epithelioretic cells in the subcapsular zone (Fig. 1).

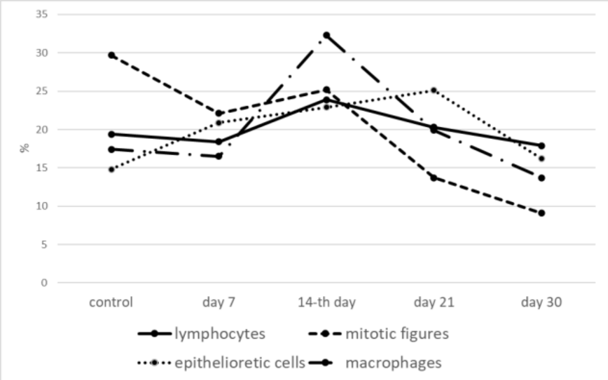

Figure 1. Cellular composition of the subcapsular zone of the cortical substance of the thymus of rats subjected to cold exposure

Table 1. Dynamics of Peripheral Blood Parameters of the Experimental Groups in Different Periods of the Experiment

Indicators, units of measure.	Control group 1, n-8 M±m	2 group day 7, n-8 M±m	3 group 14-th day, n-8 M±m	group 4 day 21, n-8 M±m	group 5 day 30, n-8 M±m
Erythrocytes, 10^{12}/l	7.58±0.43	7.24±0.41	7.54±0.98	8.44±1.52	5.45±1.93
Hemoglobin, g/l	128.35±0.95	133±6.5	132.4±14.35	132.4±24.7	123.8±22.33
Hematocrit, %	42.84±0.37	43.76±2.1	43.43±5.34	55.86±10.16	47.09±8.91
MCV, fl	56.9±0.33	60.4±0.8*	57.8±0.74	66.06±0.77*	66.36±1.33*
MSN, pg	17.31±0.1	18.42±0.36*	17.7±0.4	15.46±0.34*	17.46±0.23
MCHC, g/l	310±2.8	304±3.29	306.4±5.85	234.6±4.69*	263.8±5.05*
RDWc, %	18.34±0.15	17.72±0.54	17.72±0.54	15.94±0.45*	15.36±0.67*
leukocytes, 10^9/l	14.50±1.32	9.36±2.47	15.96±2.49	6.91±1.24*	6.99±0.62*
Lymphocytes, %	57.87±1.26	78.57±2.74*	72.97±2.60*	60.37±.15	79.75±2.15*
MID, %	14.4±1.98	11.52±1.18	15.01±0.61	7.88±1.44*	4.91±1.44*
Granulocytes, %	25.91±2.05	9.9±2.24*	13.92±2.61*	25.97±4.1	13.83±0.75*
Platelets, 10^9/l	942.85±21.5	925.6±36.8	898.8±130.5	233.6±168.7*	475.4±171.2*

Note: n is the number of observations; p is the level of statistical significance of the differences between the compared indicators, * is different from the values in the control group.

The results of a haematological examination of blood samples indicate that cold exposure affects the activity of cells that provide reactions of a non-specific and specific immune response. Thus, the total number of leukocytes decreases presumably on day 7 from the control values of 14.50±1.32 $*10^9$/l to 9.36±2.47$*10^9$/l (Table 1). At the same time, the relative number of lymphocytes increases by 35.7 %.

It was also found that stress caused by cold also affects the relative number of granulocytes (eosinophils, neutrophils and basophils). So, on the 7th day of the experiment, there was a significant decrease in granulocytes compared to the control figures (by 61 %), and the MID indicator, reflecting the content of a mixture of monocytes, eosinophils, basophils and immature cells, slightly decreases.

Indicators of the number of erythrocytes, haemoglobin concentration, hematocrit and erythrocyte indices (MCV, MCH, MCHC, RDW) did not reveal significant changes. The platelet count indicators also do not have significant differences (Table 1).

In group 3, after two weeks of exposure, the indicators of the areas of the cortical (56.0±3.16) and medulla (36.4±4.04), the cortical-cerebral index (1.55±0.23) did not differ from the indicators of the intact control.

Morphometric counting of cellular elements showed an increase in the number of lymphocytes, authentically in the subcapsular zone. There is an increase in the number of epithelial reticulocytes, as well as macrophages, the number of which has significantly changed in all areas of the thymus.

In the central zone of the cortex and the cortico-medullary zone, an increase in cells with mitotic figures, indicating their active proliferation, is noted (Fig. 2, 3).

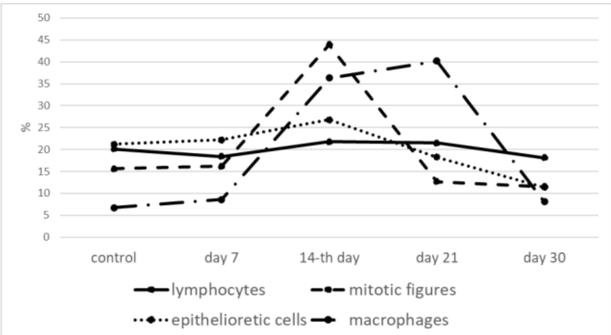

Figure 2. The cellular composition of the central zone of the cortical substance of the rat thymus under exposure to cold.

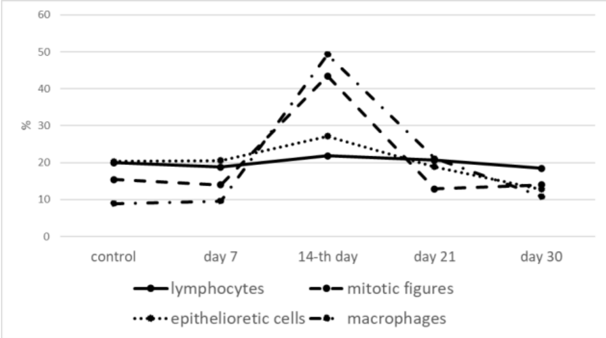

Figure 3. Cellular composition of the cortical-medullary zone of the thymus when exposed to cold

Blood parameters of the 3rd group of animals show a slight increase (10.1 %) in the total number of white leukocytes, amid a significantly increased number of lymphocytes (72.97±2.60). MID increases slightly by 2.1 %, and the number of granulocytes remains significantly reduced compared to the control group. (table 1).

After three weeks of the experiment in the 4th group, the data on the relative areas of the cortical and medullary substance were 40.2±4.76 and 48.6±8.53, the cortical-brain index (0.85±0.24) shows a significant decrease in the indicator compared to all the first groups (by 2 times, 1.5 times and 1.8 times, respectively).

The calculation of cellular elements during this period showed pronounced changes observed in all morphofunctional zones of the thymus.

In the subcapsular zone, there is a sharp decrease in the number of dividing lymphocytes by 2.1 times compared with the intact control, by 1.6 times compared with the 2nd group and by 1.8 times compared with the 3rd. At the same time, the number of epithelioretic cells is also significantly increased relative to the norm (Fig. 1).

In the central zone of the cortical substance and the cortical-medullary zones, the greatest changes are noted compared to group 3 and include a return of the indicators of dividing cells to figures close to normal and a less pronounced increase in the number of macrophages. Frequent detection of apoptotic cells and mast cells is noted.

There is an increase in the number of epithelial reticulocytes in the medulla compared to the 2nd and 3rd groups (Fig. 4).

In the indicators of peripheral blood, there is a decrease in the total number of leukocytes to the minimum values of 6.91±1.24$*$ 10^9/l during the entire experiment (Table 1). At the same time, the number of lymphocytes remains elevated throughout the experiment. The MID indicator decreases by 45.6 % by day 21.

The number of eosinophils, neutrophils and basophils on day 21 corresponds to the indicator in the control group.

In addition, there were changes in the indicators of red blood cell indices, as well as a sharp strong decrease in the number of platelets after a three-week cold exposure. The changes we observed in the peripheral blood parameters of animals are consistent with the literature data, which describe similar changes in the haematological status with a sharp decrease in the number of platelets as a reaction to stress [19, 20].

The decrease in the number of platelets is a consequence of changes in the body in response to the effects of low temperatures, since platelets take an active part in the reparative processes observed when tissue is damaged by cold, and also improve the migration of leukocytes to the centre of inflammation. Being a highly active metabolite of arachidonic acid, it is a powerful inhibitor of the aggregation of the latter. The authors also point out a decrease in the enzymatic and mediator potential of blood cells, as a result of which their ability to form aggregates decreases [21, 22].

In group 5, after one month of exposure, the indicators of the areas of the cortical (43.4±4.51), medulla (50.0±5.34) and cortical-cerebral index (0.88±0.18) remain at the same level as in group 4. Thus, according to the cortical-medulla index, there is a decrease of 1.9 times, 1.4 times and 1.7 times compared to groups 1-3.

The data of the analysis of the cellular composition of the thymuses reflect the most pronounced changes among epithelioretic cells. There is a significant decrease in their number in the subcapsular zone compared to groups 3 and 4, a significant decrease in the cortical substance and corticomedullary zone compared to all groups, and a significant increase in their number in the medulla (Fig. 4).

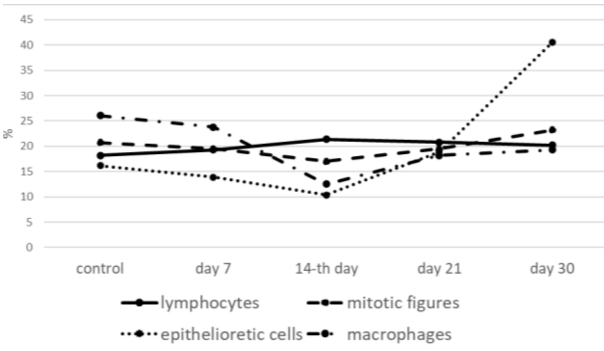

Figure 4. The cellular composition of the central zone of the thymus medulla under exposure to cold

Hassal corpuscles are characterized by pronounced degenerative changes in epithelial reticulocytes with products of cell decomposition. In the subcapsular zone, a reduced number of cells with mitosis figures is preserved, while in other zones the data do not differ from the control indicators. Similar changes in the structural and functional zones of the rat thymus during experimental cooling were noted in the works of other authors [17, 23, 24].

Structural transformations in the thymus revealed during experimental cooling for one month can be characterized as a pronounced accidental involution of the thymus at the stage of hypotrophy, the main manifestations of which are a decrease in the mass and volume of the organ, a decrease in the size of the cortical substance; suppression of lymphopoietic function and increased death of lymphocytes similar to apoptosis, ultimately leading to a decrease in the number of lymphoid population in the thymus [25, 26].

Thus, the data obtained by us indicate that exposure to the cold for 7, 14, 21 and 30 days leads to serious morphological changes that affect the state and functioning of the thymus. There is an increase in the number of lymphocytes, significant in the subcapsular zone, an increase in the number of epithelioretic cells, as well as macrophages.

In the central zone of the cortex and the cortico-medullary zone, an increase in cells with mitotic figures, indicating their active proliferation, is observed [27].

This data may indicate the development of compensatory and adaptive changes in the thymus as a response to cold exposure, pronounced on the 14th day of the experiment. There was also an increase in the number of leukocytes on day 14 (3.7% compared to the control and 59.6% compared to the indicator on day 7), which is probably due to short-term stimulation of leukocytopoiesis.

According to the obtained morphometric data, the following changes are detected on days 7, 21, 30 during the entire experiment. The volume of cortical matter in the group exposed to cold was significantly less, the volume of brain matter was larger compared to the intact group. There is a redistribution of the volume of various structural and functional zones, indicating a decrease in the functional activity of the thymus. An increase in the death of lymphocytes similar to apoptosis, a decrease in mitotic activity and the accumulation of macrophages.

When evaluating the peripheral blood parameters of experimental animals of group 5, it was found that the total number of blood leukocytes remains reduced in the value of 6.99±0.62* 10^9/l, which is not much higher than the values of group 4 (Table 1). The MID index decreases to the minimum values in this group, the number of granulocytes (eosinophils, neutrophils and basophils) also remains low. The number of lymphocytes remains significantly elevated at the level of 79.75±2.15 %. The reduced platelet level persists.

Any pathological process can affect the quantitative and qualitative features of the composition of the circulating blood. A significant decrease in the total number of white blood cells on the 7th, 21st and 30th days of the experiment indicates that exposure to low temperatures for warm-blooded animals is a stress factor. This correlates with the studies of other authors, who note a decrease in the total number of leukocytes as a result of exposure to cold, and an increase in the total content of lymphocytes [28].

Isolated immunocompetent blood cells react differently, depending on the duration of cold exposure. According to the time of exposure, the short-term cooling activates the functional activity of monocytes and neutrophils is observed, and the long-term causes suppression [20, 29]. It is noted that when rats adapt to hypothermia, indicators of nonspecific cellular immunity associated with the phagocytic activity of leukocytes react [17].

A statistically significant decrease in leukocytes and, accordingly, their phagocytic activity in both the first and second groups of animals demonstrates the suppression of nonspecific cellular immunity when exposed to low temperatures. Let's assume that the exposure of rats in our work for from 7 to 30 days, resulting in suppression of the activity of leukocytes, was long enough. Although, an increase in the number of leukocytes on day 14 (3.7 % compared to the control and 59.6 % compared to the indicator on day 7) is probably due to short-term stimulation of leukocytopoiesis. Similar results were observed in the works of K. G. Shapovalov, who established an increase in the number of white blood cells in an experiment on male Wistar rats subjected to combined (cold) stress (swimming in water at a temperature of +7 °C), mainly due to a sharp increase in granulocytes. The authors claim that as a result of the action of the cold, a specific immune response is stimulated, and the reactions of a non-specific response mediated by leukocytes are suppressed [30].

An increase in hematocrit and changes in erythrocyte indices are some of the quantitative indicators of the physiological "adjustment" of the body to the change of living conditions. An increased hematocrit with a simultaneous increase in the synthesis of erythropoietin, which activates the maturation of red blood cells, may also be one of the signs of a stress reaction, in this case to cold [31].

Also, such changes indicate a shift towards an increase in the proportion of blood cells relative to plasma, and is a reliable criterion for a stress response.

The development of thymus atrophy induced by stress, concomitant changes in peripheral blood parameters depend on the nature of the influencing factor, as well as on the duration and strength of its impact [32, 33]. Changes in the body under the influence of the cold factor include a whole complex of adaptive reactions aimed at preserving homeostasis. It is obvious that cold causes numerous adaptive reactions in the organs, responsible for hematopoiesis and immunogenesis, but finding out the dynamics of the changes taking place, the dependence of the development of immunodeficiency caused by environmental conditions and the conjugacy with the pathogenesis of several diseases is of great interest for a deeper study.

4. CONCLUSION

Thus, the effect of the cold factor causes negative structural and functional changes in the thymus in rats in the form of developing accidental involution, which manifests itself in the form of a decrease in the size of the cortical substance, inhibition of lymphocytopoietic function and increased apoptosis, leading to a decrease in the number of lymphoid populations in the thymus. An increase in the number of lymphocytes in the blood indicates that cold exposure stimulates a specific immune response. A decrease in the number of leukocytes, MID index and granulocytes indicate suppression of nonspecific cellular immunity in experimental animals.

REFERENCES

[1] N.Yu. Anokhin, The Ecological Principle of the Morphofunctional Organization of the Immune System, Hum. Ecol. 10 (2005) 39–42.

[2] H.E. Ivanova, Study of the Immune Status in Children of the Republic of Sakha (Yakutia), Med. Immunol. 19(6) (2017) 247–247.

[3] J.F. Miller, The Discovery of Thymus Function and Thymus-Derived Lymphocytes, Immunol. Rev. 185 (2002) 7–14. DOI: 10.1034 / j.1600-065x.2002.18502

[4] J.T. Wang, S. Li, J.L. Li et al., Effects of cold stress on the messenger ribonucleic acid levels of peroxisome proliferator-activated receptor-{gamma} in spleen, thymus, and bursa of Fabricius of chickens, Poult. Sci. 88(12) (2009) 2549–2554. DOI: 10.3382 / ps.2009- 00404

[5] V. Evseeva, L.K. Dobrodeeva, The Nature of the Immune Imbalance in the Indigenous People of the North of Russia, Med. Immunol. 3-4 (1999) 60.

[6] T. Isowa, H. Ohira, S. Murashima, Reactivity of Immune, Endocrine and Cardiovascular Parameters to Active and Passive Acute Stress, Biol. Psychol. 65(2) (2004) 101–120. DOI: 10.1016 / s0301-0511 (03) 00115-7

[7] E.N. Lekhanova, N.V. Golubeva, Yu.V. Romanova, Adaptive Rearrangements of the Immune Response

of the Newcomer Population of the Far North, Hum. Ecol. 5 (2007) 47–50.

[8] N.M. Kiseleva, L.G. Kuzmenko, M.M. Nkane, Stress and Lymphocytes, Nzola Pediatr. (Speransky J.) 91(1) (2012) 137–143.

[9] J.B. Hernandez, R.H. Newton, C.M. Walsh, Life and Death in the Thymus – Cell Death Signaling During T Cell Development, Curr. Opin. Cell Biol. 22(6) (2010) 865–871. DOI: org/10/1016/j.ceb2010.08.03

[10] O.V. Makarova, E.A. Postovalova, Morphological Changes in the Thymus, the Composition of Its Cells and Subpopulations of Peripheral Blood Lymphocytes in Experimental Acute Ulcerative Colitis, Bull. of Experim. Biol. and Med. 163(5) (2017) 641–646.

[11] Yu.B. Lishmanov, L.N. Maslov, A.V. Naryzhnaya et al., Endogenous Opioid System as a Link of Urgent and Long-Term Adaptation of the Body to Extreme Influences. Prospects for the Clinical Use of Opioid Peptides, Bull. of the Rus. Acad. of Med. Sci. 67(6) (2012) 73–82.

[12] A.A. Yarilin, A.D. Donetskova, T Cells – Recent Emigrants from the Thymus, Immunol. 6 (2012) 326–334.

[13] M.C. Wang-Yang, T.M. Buttke, N.W. Miller, L.W. Clem, Temperature-Mediated Processes in Immunity: Differential Effects of Low Temperature on Mouse T Helper Cell Responses, Cell Immunol. 126(2) (1990) 354–366. DOI: 10.1016 / 0008-8749 (90) 90327-n

[14] M. Banet, Long-Term Cold Adaptation in the Rat, Comp. Biochem. Physiol. A Comp. Physiol. 89(2) (1988) 137–140. DOI: 10.1016 / 0300-9629 (88) 91070-5

[15] M. Cichon, M. Chadzinska, A. Ksiazek, M. Konarzewski, Delayed Effect of Cold Stress on Immune Response in Laboratory Mice, Proc. Biol. Sci. 269(1499) (2002) 1493–1497. DOI: 10.1098 / rspb.2002.2054

[16] T. Kizaki, S. Oh-Ishi, H. Ohno, Acute Cold Stress Induces Suppressor Macrophages in Mice, J. Appl. Physiol. 81(1) (1985) 393–399. DOI: 10.1152 / jappl.1996.81.1.393

[17] V.Ya. Yurchinsky, L.A. Moreva, Comparative Morphological Study of Quantitative Ratios of Lymphocytes at Different Stages of Maturity in the Thymus of Immature Vertebrates, Med. Immunol. 19(6) (2017) 715–720.

[18] G.Yu. Struchko, E.V. Moskvichev, L.M. Merkulova et al., Morphological Picture and Immunohistochemical Phenotype of Thymus After Administration of Polyoxidonium, Immunol. 35(5) (2014) 260–263.

[19] M.A. Griggio, Thermogenic Mechanisms in Cold-Acclimated Animals, Braz. J. Med. Biol. Res. 21(2) (1988) 171–176.

[20] T. Kizaki, H. Yamashita, S. Oh-Ishi et al., Immunomodulation by Cells of Mononuclear Phagocyte Lineage in Acute Cold-Stressed or Cold-Acclimatized Mice, Immunol. 86(3) (1995) 456–462.

[21] M. Eimonte, H. Paulauskas, L. Daniuseviciute et al., Residual Effects of Short-Term Whole-Body Cold-Water Immersion on the Cytokine Profile, White Blood Cell Count, and Blood Markers of Stress, Int. J. Hyperthermia 38(1) (2021) 696–707. DOI: 10.1080 / 02656736.2021

[22] J. Hennig, U. Laschefski, H. Becker et al., Immune Cell and Cortisol Responses to Physically and Pharmacologically Induced Lowering of Body Core Temperature, Neuropsychobiol. 28(1-2) (1993) 82–86. DOI: 10.1159 / 000119005

[23] W. Savino, L.C. de Moura-Campos, G.L. Santa-Rosa, Cold as an Agent to Induce Thymic Involution in the Golden Hamster, Anat. Anz. 152(3) (1982) 239–243.

[24] C.M. Brennan, E.P. Breen, R.K. Porter, Cold Acclimation and Oxygen Consumption in the Thymus, Biochem. Biophyse Acta 1757(11) (2006) 1463–1468. DOI: 10.1016 / j.bbabio.2006.08.007

[25] S. Starskaya, V. Polevshchikov, Morphological Aspects of Thymus Atrophy Under Stress, Immunol. 5 (2013) 271–277.

[26] V.Ya. Yurchinsky, L.A. Moreva, Comparative Morphological Study of Quantitative Ratios of Lymphocytes at Different Stages of Maturity in the Thymus of Immature Vertebrates, Med. Immunol. 19(6) (2017) 715–720.

[27] A.D. Donetskova, M.F. Nikonova, N.I. Sharova et al., The Role of the Thymus in the Recovery of T-Lymphocytes After Exposure to Various Damaging Factors, Med. Immunol. 17(S) (2015) 24.

[28] L.L. Shagrov, N.A. Shutsky, S.L. Kashutin, S.I. Malyavskaya, Dynamics of Hematological Parameters of Rat Blood After Local Cold Injury, Proc. of the Karelian Sci. Center of the Rus. Acad. of Sci. 12 (2019) 86–92. DOI: 10.17076 / eb1095

[29] M.G. Novokreshchenova, O.P. Soldatova, L.A. Volkova et al., Role of Arabidopsis Thaliana nez24 Gene in Control of Response to Cold Stress, Ecol. Genet. 6(1) (2008) 20–26.

[30] K.G. Shapovalov, State of Platelets in Local Cold Lesions, Kazan Med. J. Funct. 89(5) (2008) 662–665.

[31] V.M. Safronov, Adaptive Features of Thermoregulation and Maintenance of Energy Balance in Mouse-Like Rodents, Bull. of Tomsk State Univ. 4(8) (2009) 47–61.

[32] A. Liepins, Morphological, Physiological and Biochemical Parameters Associated with Cell Injury: A Review, Immunopharmacol. Immunotoxicol. 11(4) (1989) 539–558. DOI: 10.3109 / 08923978909005384

[33] M.N. Messmer, K.M. Kokolus, J.W. Eng et al., Mild Cold-Stress Depresses Immune Responses: Implications for Cancer Models Involving Laboratory Mice, Bioessays. 36(9) (2014) 884–891. DOI: 10.1002 / bies.201400066