ANNOTATION

of dissertation work by Tokusheva Aliya Nurlanovna on the topic "Molecular and biological features of the course of aseptic inflammation associated with ecologenic immunosuppression" submitted for the degree of Doctor of Philosophy (PhD) in the specialty 6D110100– «Medicine»

The relevance of the research topic

Among medical and biological problems, the study of pathogenetic bases of inflammation is of particular importance. This is due to the development of dysregulatory shifts in inflammation in numerous functional systems, both in systems that perform an integrative regulatory role (nervous, endocrine and immune), and at the local, cellular level. This is manifested by a complex set of rearrangements in the "dynamic homeostasis" of the body, the consequence of which may be the formation of pathological conditions characterized by an expressed limitation of the functional capabilities of the body. The control and regulation of the entire complex of the aforementioned reactions is carried out by both highly specific individual and relatively autonomous mechanisms, as well as by systemic regulatory mechanisms capable of influencing a number of different organs and systems of the body. Under the influence of changing factors of the human environment (Aalami AH, Hoseinzadeh M, Hosseini Manesh P, Jiryai Sharahi A, Kargar Aliabadi E., 2022; Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR, Sadeghi M., 2021; Muhammad Imtiaz, Muhammad Shahid Rizwan, Shuanglian Xiong, Hailan Li et al., 2015), the state of body's immune system serves as an adequate indicator of a proper adaptation because immunological mechanisms subtly respond to unfavorable changes in the environment (Fortoul T. I., Rojas-Lemus M., Rodriguez-Lara V., Gonzalez-Villalva A., Ustarroz-Cano M. et al., 2014).

It is generally accepted that the physiological function of T-regulatory cells (hereinafter referred to as Treg) is important in restraining fatal autoimmune and inflammatory reactions. However, a significant knowledge gap exists related to unrecognized pathways that regulate the development and functioning of Treg cells. Likewise, we must also remember that relevant studies cannot be considered independently. Rather, they interact with each other.

From the standpoint of determining the mechanisms of immunological reactivity, it seems promising to study Tregs, whose role until recently has been associated with immune system dysfunction. It is generally accepted that Tregs are physiologically required to rstrain the aggressive autoimmune and inflammatory responses, among which CD4 + CD25 + Foxp3 + ones are the most physiologically significant. Meanwhile, the fact that regulatory cells do not act in isolation but rather have many connections with each other creating complex manipulations with the immune system should be taken into account (Zhang Xu, Xue Jiang, Xueyu Dai and Bing, 2022; Hai Zhao, Xuelian Liao, Yan

Kang, 2017). In addition, unrecognized pathways that regulate Treg development and functions still need to be identified ((Merghoub T, Wolchok JD., 2017, Ali N, Zirak B, Rodriguez RS, Cotsarelis G, Abbas AK, Rosenblum MD, et al., 2017, Arpaia N, Green JA, Moltedo B, Arvey A, Hemmers S, Yuan S, et al., 2015).

It is known that the progression of immunosuppression is often accompanied by a decrease in the number and functional activity of T-lymphocytes (Zhan Xu, Xue Jiang, Xueyu Dai and Bin Li, 2022; Bold T. D., 2011). Meanwhile, one of the main links in the pathogenesis of inflammation is lymphocytic imbalance (Th2/Th1) (Zhu J., 2015; Wang LQ, Lin ZZ, Zhang HX, Shao B, Xiao L, Jiang HG, Zhuge QC, Xie LK, Wang B , Su DM, Jin KL., 2014, Sidler C, Wóycicki R, Ilnytskyy Y, Metz G, Kovalchuk I, Kovalchuk O., 2013, Stojić-Vukanić Z, Bufan B, Arsenović-Ranin N, Kosec D, Pilipović I, Perišić Nanut M, Leposavić G., 2013). The causes of T-cell anergy in environmental immunosuppression are still unclear. It is assumed that CD4+CD25+ eTreg suppress the proliferation and activation of other subpopulations of T-lymphocytes. It is believed that this mechanism is mediated through a decrease in the production of IL-2 by T cells, which is a consequence of the intercellular interaction of Treg with an antigen-presenting cell (Troshina E.A., Senyushkina E.S., 2019; Torgashina A.V., Solovyov S. K., 2018; Shevyrev Daniil, Tereshchenko Valeriy, 2019). However, the mechanisms of Treg accumulation in inflammatory conditions that developed on the background of immunosuppression have not been studied, and so far such information has not been available in the literature.

Along with the study of systemic changes in the body during inflammation (cytokine levels, lymphocytes and macrophages count and damaged structures), it is necessary to pay special attention to tissue-specific features of gene expression. In particular, the study of the effect of gene expression on the functioning of individual components of immunity in a population living in environmentally unfavorable regions has been poorly investigated. The most promising approach is based on studying the transcriptome of tissue-specific cells. The transcriptome study will reveal the genes responsible for different degrees of activation of the immune system (Ding Wan, Jin Feng, Peng Wang, Zhenxing Yang and Tao Sun, 2022, Lin Wang, Dominik Aschenbrenner, Zhiyang Zeng, Xiya Cao, Daniel Mayr, Meera Mehta et al., 2021; Ena Oreskovic, Emily C. Wheeler, Kristen E. Mengwasser, Eric Fujimura et al., 2022). To establish the cause-and-effect relationships between mRNA expression of certain genes and changes in the activation of individual components of immunity, it is essential to study gene expression at the level of the functional activity of the entire genome, and not only individual groups of genes. The discovery of mechanisms that determine these features will be a breakthrough in understanding the pathogenesis of diseases associated with ecology, immunopathology, and inflammation; moreover, it will allow the development of fundamentally new approaches to personalized diagnosis, prevention, and treatment of environmental immune disorders.

Therefore, a scientific search in this direction cannot be limited to the immune system only. In the pathogenesis of immune disorders with the development of secondary immunodeficiency, an important role is played by the imbalance in the expression of

regulatory genes responsible for innate and adaptive immunity, which makes it difficult to manage these disorders and select the correct drug-based treatment.

All of the aforementioned gave us reason to study the features of the course of aseptic inflammation aggravated by preliminary intoxication with vanadium and chromium compounds, and also to use the newly synthesized compound as a pathogenetic management of the identified disorders.

Goal: to study the molecular and cellular mechanisms of the development of immune response in case of aseptic inflammation caused by intoxication with chromium and vanadium salts in order to create new methods for the pathogenetic correction of the identified disorders.

Problems:

1. To study the effect of chromium and vanadium on the differential expression of tissue-specific genes associated with the manifestation of inflammation;
2. To evaluate the dynamics of changes in the main immunological parameters in the peripheral blood and spleen of experimental animals with aseptic inflammation;
3. To determine the most informative immunological indicators that reflect the key mechanisms of inflammation regulation;
4. To perform an experimental assessment of the corrective effect of MXF18 on the course of aseptic inflammation in experimental rats based on the results of a quantitative assessment of splenic subcellular populations;
5. To clarify distinctive features of the course of inflammation in experimental groups on the basis of the obtained data by the method of discriminant analysis;
6. To make a pathophysiological assessment of the regulation patterns of inflammation caused by a preliminary exposure to chromium and vanadium.

Scientific novelty of the work

Heavy metal salts cause impairment of the regulatory immunological mechanisms of inflammation. For the first time, a systematic difference in gene expression in various tissues (thymus, spleen, bone marrow, mesenteric lymph nodes, and blood) of the studied groups was established.

In animals with aseptic inflammation caused by pretreatment with ammonium vanadate and potassium dichromate, 20 protein-coding genes responsible for the regulation of the immune response, including extracellular signaling proteins (cytokines) were identified for the first time. These proteins interact with cells of the immune system; they are responsible for the differentiation and proliferation of cellular components of the immune system, including adaptive immunity. By determining the ratio of regulated and unregulated genes prerequisites for downregulating the inflammatory response were established. This downregulation led to immunosuppression by inhibiting the effector and induction of the regulatory components of the immunity.

It was found that under the influence of chromium and vanadium salts, the course of aseptic inflammation was aggravated by a progressive decrease in spleen cellularity of experimental animals, as well as by inhibition of the IFNg and IL-4 activity on the

background of a decrease in the differentiation of effector T-lymphocytes, which indicated the development of T-cell anergy.

For the first time, a dysregulation of aseptic inflammation caused by pretreatment with vanadium and chromium was established. The dysregulation was explained by the induction of differentiation of splenic subcellular populations with CD4+CD25+, CD4+CD25+FoxP3+, CD4+FoxP3+CTLA+ phenotypes.

It has been established for the first time that MXF18 has an immunomodulatory effect on nonspecific resistance and immunological reactivity. For the first time, it was found that in the spleen of experimental rats, in whom the aseptic inflammation was induced by pretreatment with ammonium vanadate and potassium dichromate, MXF18 prevented the accumulation of CD4+FoxP3+ and CD4+FoxP3+CTLA4+ and prevented the intracellular accumulation of FoxP3+ throughout the experiment.

Practical significance:

1. Solving the problem of epigenetic regulation of the development of pathological conditions in the body is extremely relevant both for fundamental science and for practical application in the future. The work is aimed at solving the fundamental problem of genomics – at establishing the molecular mechanisms of epigenetic regulation of tissue-specific gene expression; revealing the influence of heavy metal compounds on the variability of the genome of immunogenesis organs and the mutual regulatory influence of important elements of these mechanisms on the course of the inflammation.
2. The significance of our exploratory research is determined by an interdisciplinary approach. The originality of the proposed approach lies in a comprehensive study of immunophenotyping of T-regulatory cells, indicators of adaptive and innate immunity with next-generation sequencing. The results obtained correspond to the world-level research; and in the future, they can be used to create algorithms that predict the nature of the course of inflammatory diseases and new therapeutic agents taking into account the environmentally unfavorable living conditions of the population.
3. The results of the study will significantly complement the understanding of pathogenetic mechanisms of inflammation by the data on the participation of gene expression in these mechanisms. The new knowledge obtained during the study will make it possible to recommend a necessary differentiated approach to anti-inflammatory therapy taking into account the phase, stage, and severity of inflammation; taking into account the activation of individual inducer genes in the early stages of inflammation, which is of great social and economic importance.

Methods of the investigation

Preclinical studies on animals have been performed in accordance with the "Rules for conducting of preclinical studies, biomedical experiments and clinical trials in the Republic of Kazakhstan" approved by order of the Minister of Healthcare of the Republic of Kazakhstan dated by July 25, 2007 No. 442 in accordance with the State Standard of the Republic of Kazakhstan "Good laboratory practice. Basic Provisions",

approved by order of the Minister of Industry and Trade of the Republic of Kazakhstan dated December 29, 2006 No. 575 and No. 557. The study took into account the recommendations set out in the "Guidelines for the Experimental (preclinical) Study of New Pharmacological Substances" edited by R. U. Khabrieva (Moscow, 2005). Also, the recommendations described in the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" (Strasbourg, March 18, 1986) have been used. All studies were performed after obtaining the decision of the Local Ethic Committee (LEC) of KazNMU (application, registration No. 166, protocol No. 3 dated 04/01/2015).

Laboratory methods

Hematological tests. The number of blood cells was counted on a hematological analyzer (Sysmex 1000i, Japan, 2010): complete blood count, which included the following parameters: hemoglobin, red blood cells, reticulocytes, white blood cells and platelets; bone marrow cytology.

Immunological tests were performed in the laboratory of M. Aitkhozhin Institute of Molecular Biology and Biochemistry in accordance with Agreement No. 34 dated 07.07.2015 with the "M. Aitkhozhin Institute of Molecular Biology and Biochemistry of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan".

Genomic data analysis. After preliminary weighing, to obtain mRNA samples, all collected organs were transported to the genomic laboratory of the B. Atchabarov Research Institute of Fundamental and Applied Medicine.

Bioinformatics analysis conducted on the basis of Agreement No. 3 of October 25, 2016 with the Institute of Bioinformatics of St. Petersburg (RF).

Statistical processing of obtained data

The data obtained during the experiments were statistically processed using the program STATISTIKA 6,0, SPSS version 15. Quantitative indicators were presented as M (SD, where M is the mean value and SD is the standard deviation).

To check the coincidence of the distribution of studied quantitative indicators with the normal distribution in the groups, the Kolmogorov-Smirnov test was used. If the law of distribution of the studied numerical indicators differed from the normal one, then the statistical significance of differences was tested using the Mann-Whitney U-test (in the case of paired independent samples) and the Kruskal-Wallis test (in the case of multiple independent samples). Differences were considered significant at $p < 0,05$. To determine the existence of functional relationships between parameters, the R Spearman correlation coefficient was calculated, which was considered significant at $p < 0,05$. Genomic data analysis was performed with R, packages DESeq2 and Reactome.

Research object:

The experiments have been performed on 220 outbred male rats with an average weight of 180-240 g.

Experimental studies have been performed in the following sets:

Set 1: sexually mature rats (control);
Set 2: sexually mature rats with aseptic inflammation;
Set 3: sexually mature rats + ammonium vanadate (AV) and potassium dichromate (PD);
Set 4: sexually mature rats + metals + aseptic inflammation (experiment);
Set 5: sexually mature rats with aseptic inflammation + MXF18;
Set 6: sexually mature rats with aseptic inflammation + PO;
Set 7: experiment + MXF18;
Set 8: experiment + PO.

Animals from sets 2-8 have been divided into 3 subgroups of 10 rats each.

Aseptic inflammation was modeled by subcutaneous injection of 0,3 ml of turpentine in vaseline oil into the interscapular region of rats. Prior to doing that, the hair in the interscapular region was cut off and 0,5 ml of air was injected subcutaneously.

Combined inoculum containing ammonium vanadate and potassium dichromate has been prepared daily (except for Sundays) for two weeks at a dose of 5 mg/kg of body weight orally with a metal probe. Immediately after a two-week inoculation of AV and PD in rats of the second and fourth sets of the experiment, aseptic inflammation has been modeled. The slaughter of all animals has been carried out after the 1, 7, and 14 days from the beginning of the modeling of aseptic inflammation. After slaughter, the thymus, spleen, mesenteric lymph nodes, bone marrow and blood were taken from all rats for immunological, hematological, and genomic studies. Animals were weighed before and after the experiment. The collected organs were weighed before their transportation to the laboratory of M. Aitkhozhin Institute of Molecular Biology and Biochemistry and the genomic laboratory of the B. Atchabarov Research Institute of Fundamental and Applied Medicine. In the journal of experimental registration protocols, all the obtained parameters were recorded, as well as visual indicators of the state of the internal organs of the experimental rats. In addition, photo and video filming of the experiment were carried out.

The main provisions of the thesis submitted for its defense:

1. Aseptic inflammation induced by ammonium metavanadate and potassium dichromate causes dysregulation of tissue-specific genes, which predicts an unfavorable outcome of the inflammatory process.
2. Vanadium and chromium complicate the course of experimental inflammation by the development of anemia, deficiency of leukocyte immunity and cytokine regulation, as well as impaired differentiation of T- and B-cell immunity.
3. Key mechanisms of vanadium- and chromium-induced immunosuppression are associated with impaired cellular and humoral immunity.
4. MXF18 has an immunomodulatory effect; and while used, it selectively neutralizes the immunotoxic effects of vanadium and chromium compounds as evidenced by hematological and immunological parameters of blood and spleen of experimental animals with aseptic inflammation.
5. The effectiveness of MXF18 at different times of the experimental study is selectively comparable with polyoxidonium.

Research results

Differential gene expression was determined using the DESeq2 package. Nine comparisons were processed but only thymus caused persistent differences in expression at the gene level. As a result of the bioinformatics analysis, 20 genes were identified. Separate analysis for upregulated showed 10 significantly enriched gene sets from the GOBP category. Thus, 11875 regulated genes were enriched for 10 GOBP categories (FDR <0.05), including the process of cellular response to stress (1,565 genes, of which 140 genes overlap with this category, FDR=1.07E-44).

There was enrichment of 1,893 regulated genes for 3 canonical pathways (cell cycle, immune system and adaptive immune system) (237 gene overlaps, FDR=5,17E-24). Based on the analysis of gene modules using the MSigDB database and gene set enrichment analysis (GSEA), it was found that metal compounds cause disturbances in signaling and metabolic pathways. Thus, metals activate the genes involved in the cell cycle in the compared groups: AI versus Me + AI and versus Me. The carried out study revealed differentially expressed genes with an average strength of expression (up-down), which were differentially regulated in the thymus. Twenty protein-coding genes were identified: Tnfrsf14, Cr2, Sp3, Stag2, H3f3a, Dpp4, Anp32a, Prpf40a, Hsp90aa1, Tardbp, Dnaja1, Eef2k, Mfge8, Macf1, Arhgef11, Txndc5, Srrm2, Syk, Clu, Cd19.

As it is known, the course of the inflammatory process is accompanied by the inclusion of immunological mechanisms of body defence, mainly due to the cellular components of the blood. Therefore, in 7 days in the AI group, a statistically significant increase in total white blood cells by 69,7% was observed compared to the control, which was facilitated by an increase in neutrophil and lymphocyte absolute counts by 153,3% and 59,1%, respectively. In 14 days, these indicators remained above the control level.

A distinctive feature in the blood of the Me + AI group was the lower count of white blood cells. Thus, the values of total white blood cells remained at the control level, while the absolute lymphocyte count was lower than in AI by 58,1% (p=0,0076) in 7 days and by 29,7% (p=0,0245) in 14 days.

In the comparative characteristics of the neutrophil count for AI and Me + AI groups, the following tendency was noted: the neutrophil count in the Me + AI group significantly exceeded the control values only for 60% and 86,7% but it did not reach the neutrophil count in AI.

The decrease in monocytes on the 1st day of the study in the Me + AI group was replaced by their return to the control level on the following day; while for the AI group, the 14th day of the experiment was accompanied by a statistically significant increase in monocytes by 83,3% compared to the control (p = 0,0019). The statistically significant decrease in RBCs in AI and Me + AI groups in 7 days from the beginning of the experiment (p=0,0083, p=0,0856, respectively), was replaced by their return to the control level in 14 days. Meanwhile, the hemoglobin levels in all groups remained significantly lower than the control in all periods of the study. Preliminary administration of heavy

metal salts in Me and Me + AI groups caused the reduction of the average volume of hemoglobin in RBCs, which was assessed on the 14th day by significantly lower MCH values (6% и 4,1%, respectively) while compared to the control group. The injection of animals with heavy metal salts had absolutely no effect on the level of IL-6. At the same time, aseptic inflammation was accompanied by a 6-fold increase in the cytokine level on the day 1 of observation *(M=273, SD=28 pg/ml, p_k=0,004)*, which returned to normal in subsequent periods (7 days, 14 days). However, the development of aseptic inflammation induced by the injection of heavy metal salts reduced the release of IL-6 into the bloodstream on the 1st day of observation *(M=144, SD=24 pg/ml, p_{AI}=0,004)* with normalization of the cytokine level in subsequent periods. Exposure to Me inhibited the production of IL-6 on the 1st day.

The level of IL-1β did not change significantly in all variants of the experiment. The level of TGF β did not differ significantly from the control (61 ± 7 ng / ml) when exposed to Me or AI but significantly increased *(M = 80, SD = 6 ng / ml, p_k = 0,023, M = 75, SD=5 ng/ml, P=0,05, respectively)* with their combined effect on the 7th and 14th days of epy observation. There were no significant changes in the level of IL-10 in all the studied groups, except for the Me + AI group. In animals of the Me+AI group, on the 1st day of the observation, there was a significant increase in the content of IL-10 in peripheral blood (M=142, SD=±24 pg/ml, p_k=0,013). In subsequent periods of observation, the level of IL-10 returned to normal and did not exceed the average level in the control (M=23, SD=2 pg/ml).

The results clearly demonstrate that under the influence of vanadium and chromium compounds, spleen cellularity starting from the 7th day of the experiment decreased and did not recover until the end of the experiment. Thus, the dynamics of changes in spleen cellularity looked as follows: exposure to salts of heavy metals in a day after the last injection already led to a more than twofold decrease in spleen cellularity compared to the control (from 0,78±0,2 million cells/mg to 0,31±0,03 5 million cells/mg, p=0,002). This indicated the cytotoxic effect of heavy metal salts on the spleen. In 7 days, a statistically significant (p=0,007) increase in spleen cellularity up to 0,5±0,05 million cells/mg was observed, which can be explained by the development of a compensatory reaction of the spleen in response to the toxic effects of heavy metals. However, this splenic hyperproliferation turned out to be transient because since the 14th day the cellularity value (0,48±0,02 million cells/mg) was similar to one on the 7th day of the experiment, and a statistically significant difference compared to the control (p=0,01) remained. Potentially, there was an exhaustion of compensatory proliferative processes in spleen.

A distinct picture was observed in case of administration of turpentine, which induced subcutaneous aseptic inflammation. In 7 days, we did not observe a difference in this

parameter compared with the control, while in 14 days the spleen cellularity significantly decreased to 0,47±0,08 million cells/mg *(p=0,01)*, which indicated the induced inflammation in the model. The combined effect of heavy metals and inflammation on the spleen was characterized by a sharp decrease in cellularity to 0,48±0,05 million cells/mg *($p_{control}$=0,01)* in 7 days; and 0,5±0,06 million cells/ mg *($p_{control}$=0,02)* in 14 days of the experiment, which did not differ from the effect of metals alone. Thus, vanadium and chromium salts are able to reduce the cellularity of the spleen of experimental rats with aseptic inflammation.

The next direction of our research was the populations of T- and B-lymphocytes. The experiments showed that the proliferative activity of CD3 + CD4 + T-lymphocytes remained at the level of control values during the week (Fig. 3.21). After 14 days in the AI group, the proportion of CD3+CD4+ increased by 23,2% *(M=31,3, SD=8,7; p7day=0,014)* compared with the previous study period *(M=25,4, SD=3,8)*. Meanwhile, during this period of the study in the Me and Me+AI groups, the proportion of CD3+CD4+ significantly lagged behind AI, respectively, by 8% *(M=28.8, SD=3; pAB=0.002)* and 28,4% *(M=22,4, SD=4,6; pAI=0,008)*.

In the AI group, for Th1 subpopulations, a statistically significant increase in IFNg was established both after the day 1 by 29,3% and after the day 7 and 14 by 95% and 63,1%, respectively. The level of detectable IL-4 (Th2) after the days 1 and 7 of the study exceeded the control values by 41,4% and after 14 days by 76%. A different picture was found in a group of rats inoculated with salts of vanadium and chromium. Thus, in the Me group, vanadium and chromium salts caused consistently low and, accordingly, different from the control production of IFNg and IL-4 during all periods of the study. The development of inflammation in Me + AI group caused the preservation of IFNg and IL-4 at the control level in the first period of the study, while their content lagged behind the similar group of AI, not exposed to vanadium and chromium salts, respectively, by 25% ($p \leq 0,05$) and 17,1%. In subsequent periods, the content of IFNg and IL-4 progressively lagged behind the AI values by 68,6% and 73,2% in 7 days and by 66% and 76,5% in 14 days, respectively.

The content of CD8+ for AI was 1,3 times ($p \leq 0,05$) significantly higher compared to the control after the 1 and 14 days of the study. In the Me+AB group in 14 days, the content of CD8+ significantly lagged behind the AI values by 29%. The development of aseptic inflammation in the AI group did not affect the proliferative activity of B-lymphocytes either in 7 or 14 days of the study.

A downward trend in the proportion of CD45+B220+ splenocytes was observed in 7 days in the group of pretreated animals (Me) (M=40,5%, SD=7,8 versus M=44,5%, SD=9,1 in the control group), which by the 14th day of the experiment reached significant

differences both in relation to the control (M=35,5, SD=4,8 versus 44,5, SD=9,1; $p_{control}$=0,0003) and in relation to AI (M=45,3, SD=2,5, p_{AI}=0,01).

Combined exposure to metals and induced aseptic inflammation significantly reduced the proportion of CD45+B220+-splenocytes relative to the control by 33,1% (M=29,8, SD=2,8 versus M=44,5, SD=9,1 control, $p_{control}$=0,009) in 7 days and by 57,1% (M=19,1, SD=5,1 versus M=44,5, SD=9,1 control, $p_{control}$=0,0003) in 14 days. At the same time, preliminary injection of rats with metal salts led to a decrease in this indicator and compared with group AI in 7 and 14 days, respectively, by 33,5% (M=29,8, SD=2,8 versus M=44,8, SD=4,2 AB, p_{AB}=0,00008) and by 60% (M=19,1, SD=5,1 versus M=45,3, SD=2,5 AB, p_{AI}=0,00007).

In response to antigenic stimulation of the inflammatory focus, rats of the Me and Me + AI groups showed an increase in the expression of RT1+, by 2,7 *(M=55,0; SD=15,3 vs. M=20,6; SD=11,8 control, $p_{control}$ =0,007)* and 2,2 times *(M=46,3; SD=15,3 versus M=20,6; SD=11,8 control, $p_{control}$=0,016)* respectively, on the 7th day of the experiment in relation to the control. Moderate accumulation of the proportion of RT1+ in CD45+CD45R(B220)+ gate in rats of the AI group had no statistically significant difference with the control one. By the 14th day of the experiment, for this group of animals, the expression of RT1+ decreased to the control level, which apparently indicated a decrease in antigenic stimulation from the focus of inflammation. Meanwhile, by this time of the study, the expression activity of RT1+ in Me (M=62,0; SD=6,2, $p_{control}$ =0,0001) and Me+AI (M=62,8; SD=6,8, $p_{control}$ =0,0001) groups reached the same level, which was 3 times higher than the control values (M=20,6; SD=11,8). A similar difference was achieved in the Me + AI group and in relation to the AI.

The activity of B-cells in the experimental groups was assessed by the expression of the MHC-II molecule analyzing the proportion of B220+RT1(MHC-II)+ splenocytes in CD45+ lymphocytes gate. On the 7th day after the induction of aseptic inflammation, a statistically significant increase in B220+RT1+ splenocytes *(M=28,2; SD=9,3; $p_{control}$=0,15)* was observed compared with control values *(M=14,7; SD =5,1)*, which indicated a normal pro-inflammatory background. On the 14th day, this indicator had values comparable to the control ones. In the Me group, the number of B220 + RT1 + splenocytes compared with the control was increased after 7 days of the experiment *(M = 25,4, SD = 5,2; $p_{control}$ = 0,044)*, which may be due to the stimulating effect of vanadium compounds on the proliferation of B-lymphocytes shown earlier in experiments with sodium metavanadate ($NaVO_3$). The proportion of B cells expressing RT1+ in the Me group after 14 days of the experiment was 2,5 times *(M=28,8, SD=3,0; p_{AI}=0,003)* compared with AI *(M=11,4, SD = 8,7)* and significantly higher *(M=28,8, SD=3,0; pMe+AI=0,025)* for 1,5 times compared with Me+AI *(M=18,9, SD=6,6)*.

The proportion of splenocytes with Treg cells (CD4+CD25+) phenotype did not change in the AI group within 7 days from the onset of aseptic inflammation, and on the 14th day, there was a more than two-fold increase in these cells *(M=5,5, SD=0,8; $p_{control}$=0,001)* compared with the control *(M=2,3, SD=0,4).*

In contrast, in the Me and Me+AI groups, on the 7th day of the study, an increase in the proportion of CD4+CD25+ from the control level *(M=2,3, SD=0,4)* took place respectively, by 87% *(M=4,3, SD =0,8; $p_{control}$=0,03)* and 100% *(M=4,6, SD=0,3, $p_{control}$<0,0001)*. The upward trend continued on the 14th day, where the difference with the control was 91,3% for Me *(M=4,4, SD=1,7; $p_{control}$=0,033)* and 156,5% *(M =5,9, SD=1,2; $p_{control}$=0,001)* for Me+AI.

The transcription factor Foxp3 is critical for the development and function of regulatory T-lymphocytes. Expression of FoxP3, which plays a major role in the suppressor activity of Treg cells, was analyzed in the $CD4^+CD25^+$ gate and the suppressor molecule CTLA-4 by $CD4^+FoxP3^+$ splenocytes had similar dynamics in the AI group in 14 days, which indicates the positive role of the expansion of the pool of Treg cells in the resolution of inflammation. Thus, in 7 days in the AI group, the proportion of FoxP3 expressing $CD4^+CD25^+$ *(M=14,4, SD=3,2)* and $CD4^+FoxP3^+CTLA4^+$-splenocytes *(M=12,3, SD=5,0)* fluctuated at the level of the corresponding controls *(M=12,0, SD=4,0; M=11,6, SD=2,6)*. Only by the 14th day of the study a pronounced increase in the activity of $CD4^+CD25^+FoxP3$ and $CD4^+FoxP3^+CTLA4^+$ was observed by 2,3 times (M=28,1, SD=6,2; pcontrol<0,001) and 1,8 times *(M =20,8, SD=6,4; pcontrol=0,015)* respectively compared with the control. At the same time, we observed an increase in the proportion of $CD4^+CD25^+$-splenocytes and the expression of FoxP3 by these cells in Me group as compared with the control on the first day after the end of Me administration; this persisted until the end of the experiment. In 7 days, the proportion of $CD4^+CD25^+FoxP3$ significantly exceeded the control level by 58,3% *(M=19,0, SD=3,2; $p_{control}$=0,041)* and continued to increase on the 14th day, exceeding the control for 2 times *(M=24,0, SD=4,0; $p_{control}$=0,021)*. A similar picture was observed in the Me+AI group. Expression of CTLA-4 by $CD4^+FoxP3^+$-splenocytes in the Me and Me+AI groups significantly increased on the 7th day by 2 times (M_{Me}=21,5, SD=1,3; M_{Me+AI}=21,0, SD=5,0; $p_{control}\leq$0,05) and remained until the end of the experiment.

The results of studies of hematological blood analysis in rats with aseptic inflammation treated with MXF18 and PO. On the 7th day after modeling the aseptic inflammation and the correction of MXF18 and PO, we found an increased level of WBC, lymphocytes, neutrophils and monocytes, which was 2-3 times significantly higher than similar indicators in the AI group. The 14th day of the study was characterized by a one-and-a-half to two-fold decrease in the studied parameters in the AI+ MXF18 and AI+PO groups from the previous level, but exceeding the AI indices. There were no statistically

significant differences between AI+ MXF18 and AI+PO. The conducted studies have established that MXF18 and PO did not restore the indicators of RBCs, hemoglobin and hematocrit, which in all periods of the study turned out to be significantly lower than the control level.

The study of spleen cellularity showed that after 7 days under the influence of PO and MXF18, spleen cellularity did not reach the level of control and AI. After 14 days, spleen cellularity in the AI+PO group continued to decrease, while MXF18 restored spleen cellularity *(M=0,7, SD=0,1)*, exceeding the AI value by 40% *(M=0,5, SD=0,1; $p_{AI+MHF18}$=0,002)*; AI+PO by 75% *(M=0,4, SD=0,1; $p_{AI+MHF18}$=0,0008)* and also their own values of the previous period by 16,7% *(M=0,7 , SD=0,1 versus M=0,6, SD=0,1; p_{7day}=0,034)*.Thus, by the 14th day of the study, MXF18, compared with PO, restores the spleen cellularity, returning its values to the control level.

The conducted studies showed that polyoxidonium did not stimulate the expansion of the B-cell pool during the week, and the CD45+B220+ values were lower than the AI level by 25,3% (pAI=0,024). The proliferative activity of CD45+B220+ splenocytes under the influence of MXF18 in this study period exceeded the values of AI+PO by 42% (pPO=0,011). In 14 days, the AI+PO values returned to the control level, while MXF18 significantly reduced CD45+B220+ about the control by 34,8% ($p_{control}$=0,006), by 36% (p_{AI}=0,00008) to AI, by 32,4% (p_{PO} = 0,0008) to AI + PO; and to own values of the previous period by 38,8% (p_{7day} = 0,00008). MXF18 reduces antigenic stimulation from the focus of inflammation, which contributed to the inhibition of CD45+B220+ proliferative activity.

The percentage of active B-lymphocytes capable of expressing the MHC-II molecule 7 days after the application of PO and MXF18 fluctuated at the level of AI, exceeding the control level by 56,5% *($p_{control}$ = 0,018)* and 49,6% *($p_{control}$ = 0,016)*, respectively. In 14 days under the influence of MXF18, the proportion of B220 + RT1 remained at the level of the previous study but significantly exceeded the control, AI and AI + PO groups, by 84,4% ($p_{control}$ = 0,005), 137,7% (p_{AI} = 0,006) and 204,5% (p_{AI+PO}=0,0004) respectively.In 7 days, RT1+ expression in activated B-lymphocytes significantly increased under the influence of PO, the values of which were 48% and 141,3% higher than AI *(p_{AB}=0,041)* and control *($p_{control}$=0,0008)*, respectively. In 14 days, MXF18 stimulated the expression of RT1+, the values of which were 2,7 times higher than the control *($p_{control}$=0,0002)*; 3,1 times than AI (p_{AI}=0,006), 4,6 times than AI+PO *(p_{AI+PO}<0,05)* and the proper values of the previous period by 1,5 times (p_{7days}=0,003).In 7 days, the proportion of CD4+CD25+ splenocytes under the influence of MXF18 *(M=3,9, SD=0,9)* significantly exceeded the values of groups AI *(M=2,1, SD=0,9)* and AI+PO *(M=2,6, SD=1,0)* by 85,7% *(p_{AI}=0,005)* and 50% *($p_{AB+MXF18}$=0,051)*, respectively. In 14 days, under the influence of MXF18, the tendency towards an increase in CD4+CD25+ splenocytes was insignificant.

Meanwhile, these values increased significantly in groups AI *(M=5,5, SD=0,8)* and AI+PO *(M=5,5, SD=1,3),* exceeding the proper values of the previous period by 2,6 *($p_{7\,day}$<0,0001)* and 2,1 times *(p_{7day}=0,002)*.

As a result of our studies, it was found that in 7 days the proportion of CD4+FoxP3+ under the influence of MXF18 *(M=3,2, SD=0,5)* was 60% (p_{AI+PO}=0,025) more than PO (M=2,0, SD=0,5). In 14 days, there was a significant increase in suppressor activity in the AI and AI+PO groups. Thus, in the AI group, the proportion of CD4+FoxP3+ increased by 82,6% compared to the control *(M=2,3, SD=0,2) (M=4,2, SD=0,5; $p_{control}$<0,0001)*, and in the AI+PO group by 126,1% *(M=5,2, SD=1,3; $p_{control}$=0,002),* which exceeded the proper results of the previous study *(M=2,0, SD=0,5)* for 2,6 times *(p_{7day}=0,001)*. Meanwhile, the proportion of CD4+FoxP3+ in the AI+ MXF18 group during this period of study *(M=2,8, SD=0,9)* fluctuated at the level of the previous period, but turned out to be two times less than the AI+PO group *(p_{AI+PO}= 0,006)*.

The study of CD4+ T-regulatory cells expressing FoxP3+ showed that in 7 days their proportion under the influence of MXF18 *(M=19,7, SD=1,4)* exceeded the control *(M=12,0, SD=2,3)* by 64,2% *($p_{control}$=0,0006)*, AI *(M=14,4, SD=4,0)* by 36,8% *(p_{AI}=0,022)*, AI+PO *(M=14,6, SD=2,8)* by 35% *($p_{AI\ +\ PO}$ = 0,008)*. In 14 days in the AI+ MXF18 group, the proportion of FoxP3+ cells in the CD4+CD25+ lymphocyte gate returned to the control level. Subsequently, the proportion of CTLA4+ expressed in the CD4+FoxP3+ lymphocytes gate and those changed under the influence of MXF18 and PO was studied. Their proportion sharply increased after 7 days, while on the 14th day of the study, a decrease in expression activity was observed. Thus, the proportion of CTLA4+ in the CD4+FoxP3+ lymphocytes gate under the influence of MXF18 *(M=25,3, SD=3,0)* increased by 118,1% ($p_{control}$=0,004) relative to the control *(M=11,6, SD =2,6)* and 105,7% *(p_{AI}=0,002)* more compared to AI *(M=12,3, SD=5,0)*. Similar results were obtained under the influence of PO.

On a day of correction with MXF18 and PO, the average value of WBCs and lymphocytes was significantly higher by 94,7% and 94,4%, respectively than the experimental values. After a week of correction with MXF18, the increase in blood parameters continued, and their values differed from the control and experiment, respectively, by 2,1 and 2,6 times for WBCs, by 2,1 and 3,2 times for lymphocytes and by 1,7 and 2,5 times for monocytes. The neutrophil count exceeded the one in the control and the previous period by more than 1,5 times. Statistically significant differences between MXF18 and PO were observed during the week of the study when the effectiveness of MXF18 exceeded the one of PO by 69,4% in relation to leukocytes, by 67% in relation to lymphocytes and by 100% in relation to monocytes.

Further studies aimed to evaluate the corrective efficacy of MXF18 and PO on the immunophenotypic parameters of splenic populations of lymphocytes in experimental animals. Thus, 7 days after modeling aseptic inflammation in experimental animals under the influence of MXF18, the proportion of B cells *(M=36,2, SD=5,6)* was 21,5% higher compared to the Experimental group (M=29,8, SD=2,8;) (pPO=0,040). The proportion of B220+RT1+ after exposure to MXF18 and PO remained at the level of untreated animals.

Expression activity of B-lymphocytes ("% RT1+ in CD45+CD45R(B220)+ gate" column) under the influence of MXF18 *(M=51,8, SD=7,6;)* and PO *(M=47,2, SD=11.6;)* increased exceeding the control level by 151,4% ($p_{control}$=0,006) and by 129,1% ($p_{control}$=0,05), respectively. However, the same values corresponded to the experiment group. In both 7 and 14 days, a statistically significant increase in the proportion of B cells under the influence of MXF18 was observed. Thus, the proportion of CD45+CD45R(B220)+ ("number of B-cells" column) in the Experiment + MXF18 group *(M=31,1, SD=6,0)* increased by 62,8% more (experiment=0,006) if compared with the experimental group. Similar results were obtained for PO. In 7 days, the determination of the surface expression of 4 clusters of differentiation (CD4+, CD4+CD25+, CD4+CD25+FoxP3+, CD4+FoxP3+CTLA4+) in each group showed that both drugs affected the immunophenotype of lymphocytes in the same way by reducing the expression of CD4+CD25+, CD4+CD25+FoxP3+, CD4+FoxP3+CTLA4+ compared with the Experience group. Thus, under the influence of MXF18 and PO, the expression of CD4+CD25+ was 3,7% *(SD=0,6)* and 3,4% *(SD=0,4)*, respectively, which was lower than the values for Experience group *(M=4,6, SD = 0,3)* by 19,6% *(exp = 0,017)* and 26,1% *(exp = 0,0003)*. The expression of CD4+CD25+FoxP3+ decreased under the influence of MXF18 *(M=11,6, SD=4,6)* by 42% *(exp = 0,036)* and under the influence of PO *(M=6,9, SD=3,7)* by 65,5% *(experiment=0,005)* compared with experiment *(M=20,0, SD=6,1)*. The expression of CD4+FoxP3+CTLA4+ both under the influence of MXF18 and under the influence of PO was 2 times significantly lower than in the experimental group. Differentiation of CD4+CD25+ cells in the pool of CD4+ T-lymphocytes under the influence of MXF18 and PO remained the same as after 7 days of research. Meanwhile, a significant increase in the expression activity of CD4+CD25+FoxP3+, CD4+FoxP3+CTLA4+ lymphocytes under the influence of MHF18 and PO was observed. Thus, it was found that after 14 days, under the influence of the PO, the expression of CD4+CD25+FoxP3+ *(M=20,1, SD=4,3)* exceeded the previous expression level *(M=6,9, SD=3,7)* and the control level *(M=11,9, SD=2,3)* by 191,3% *(p_{7days}=0,0007)* and 68,9% *($p_{control}$=0,0007)* respectively but remained in the range of experimental values.In 14 days, under the influence of MXF18, there was a slight increase in CD4+CD25+FoxP3+ *(M=15,6, SD=6,6 versus M=11,6, SD=4,6)*, which was 34,5% higher than the proper values of the previous term *(p_{7days}=0,0007)*, but their level lagged behind the experimental ones by 1,6 times *(M=24,7, SD=3,3; exp=0,019)*. In 7

days, the proportion of CD4+FoxP3+CTLA4+ lymphocytes was almost 2 times below the experiment *(M=21,0, SD=5,0)* both under the influence of PO *(M=10,1, SD=3,0)* and MXF18 *(M=11,1, SD=3,5; exp<0,05)*. In 14 days, there was an increase in the proportion of CD4+FoxP3+CTLA4+ both in relation to the control and to the previous period under the influence of PO *(M=18,2, SD=3,8 vs. control: M=11,6, SD= 2,6 versus 7 days; M=10,1, SD=3,0)* by 56,9% *($p_{control}$=0,014)* and 80,2% *(p_{7day}=0,006)* respectively; as well as under the influence of MXF18 *(M =25,4, SD=9,3 versus control: M=11,6, SD=2,6, vs. 7 days; M=11,1, SD=3,5)* for more than 2 times *($p_{control}$ =0,014, p_{7days}=0,012)*.

Conclusions:

1. Analysis of differentially expressed genes showed group differences in the expression of thymus genes in the "Me + AI" versus "AI" groups, as a result of which 20 protein-coding genes were identified. Overexpression of Hsp90aa and Stag2, responsible for the translation of immunosuppression signals, as well as statistically significant increase in the expression of Tnfrsf4, Clu, Sp3, Cd19, which trigger the activation of Tregs, FoxP3, apoptosis, chronic inflammation, and tumor growth have been established. A statistically significant decrease in the expression of innate immune regulators (Dpp4, Txndc5, Syk) responsible for the expression of cellular inflammatory cytokines (IL-6, TNF-α), apoptosis and B-cell differentiation was found, which is a predictor of an unfavorable course of inflammation.

2. Key indicators of experimental inflammation under the influence of ammonium metavanadate and potassium dichromate in the blood of experimental animals were associated with the: development of anemia; WBCs deficiency (mainly due to low lymphocyte and neutrophil counts); imbalance of cytokine regulation towards increased anti-inflammatory IL-10 and TGF-β cytokines and insufficient production of IL-6 and IL-1β; imbalance of splenic subcellular populations with IFNg+/IL-4- and IFNg-/IL-4+ phenotypes; impaired differentiation and subsequent activity of B-lymphocytes, induction of differentiation of splenic subcellular populations with CD4+CD25+, CD4+CD25+FoxP3+, CD4+FoxP3+CTLA+ phenotypes.

3. The greatest contribution to the characterization of vanadium- and chromium-induced disorders in the manifestation of inflammation was made by the development of anemia, a disorder of the humoral immunity and nonspecific resistance of the body with the dominance of the suppressor role of splenic subpopulations of T-regulatory lymphocytes.

4. It was found that within a week of the study, the corrective efficacy of MXF18 exceeded the PO in relation to blood WBC fractions. The corrective efficacy of MXF18 and PO in relation to RBCs and hemoglobin was not established within the indicated periods of study. MXF18 increases the proportion of B cells, among which there was a significant increase in cells with the B220+RT1+ phenotype. However, PO, as well as MXF18 did not change the proportion of RT1+ in the

CD45+CD45R(B220)+ lymphocyte gate of experimental rats. Comparability of the expression levels of immunosuppression markers on the surface of lymphocytes during vanadium and chromium salts-induced inflammation under the influence of MHF18 and PO led to an increase in the expression of immunophenotypic markers CD4+CD25+FoxP3+, CD4+FoxP3+CTLA4+ by the 14th day of the study, which contributed to resolution inflammatory process.

5. As a result of the discriminant analysis, it was possible to establish that the groups "Experiment + MXF18" and "Experiment + PO" discriminate with the group "Experiment" in terms of indicators characterizing the degree of inhibition of activation of CD4+FoxP3+ and CD4+FoxP3+CTLA4+. From those above, it follows that on the 7th day of the course of aseptic inflammation, MXF18 and PO equally prevent the accumulation of CD4+FoxP3+ and CD4+FoxP3+CTLA4+. On the 14th day of the study, it was found that MXF18 discriminates with PO in terms of its effectiveness in preventing the intracellular accumulation of FoxP3+ throughout the experiment. In addition, unlike PO, MXF18 better stimulated the proliferative activity and, thus, the phagocytic activity of monocytes with an increasing effect throughout the experiment.

6. Transcriptomic analysis of tissue-specific gene expression gave a fundamental insight into the patterns of regulation of the immune response under conditions of intoxication with chromium and vanadium salts. Our results demonstrated different thymic gene expression profiles of high and low-response experimental rats during acute aseptic inflammation. More genes with reduced modulation disrupted the inflammation resolution mechanism. Among the 20 identified genes, Hsp90aa (heat shock protein) played a key role as an initiator of the suppressive immune response. The conducted fundamental studies were consistent with the conclusions of transcriptomic analysis and confirmed their role in regulating inflammatory phenotypes. This was confirmed by comparing the results of quantitative and qualitative assessments of inflammatory phenotypes with the expression profile of the identified genes.

Practical significance

1. Data obtained in the course of experimental studies can be useful in conducting applied research devoted to the study of medical problems within the framework of the corresponding priority area.
2. Data on the immunotoxic effects of vanadium and chromium, as some of the common anthropogenic pollutants, can be useful in assessing risk factors for the disease, taking into account the region of residence of the patient and preventing its chronicity, including an assessment of the immune status in the diagnosis.
3. The findings of this study may also be useful for government and commercial structures interested in the development and promotion of new domestically produced medicinal brands.

4. The dissertation materials can be used in the educational process at medical and biological faculties, as well as in postgraduate education courses for immunologists, therapists and general practitioners.
5. Based on the results of the research, two utility model certificates were obtained. A monograph was published based on the dissertation materials, which is included in the list of recommended literature for 2nd and 3rd year students of general medicine in preparation for practical training.

Approbation of the research results.

The main provisions of the thesis were reported at the meetings of the Department of Pathophysiology of non-commercial JSC "S.D. Asfendiyarov KazNMU".

The main provisions of the thesis were reported and discussed at:

1. International scientific and practical conference "Relevant scientific research in the modern world" (Pereiaslav-Khmelnytskyi, Ukraine, October 26-27, 2016)
2. "8th European Immunology Conference 2017" (Madrid, Spain, June 29-July 1, 2017)
3. ISER "International Conference on Science, Health and Medicine" 2017 (Athens, Greece, November 7-8, 2017)

Papers published on the results of thesis:

1 article indexed in the Scopus and PubMed information databases:

1. Marina K. Balabekova, Yekaterina O. Ostapchuk, Yuliya V. Perfilyeva, **Aliya N. Tokusheva,** Adilman Nurmuhambetov, Rustam R. Tuhvatshin, Vasiliy V. Trubachev, Zhaugashty B. Akhmetov, Nurshat Abdolla, Gulgul K. Kairanbayeva, Koks Sulev & Nikolai N. Belyaev. Oral administration of ammonium metavanadate and potassium dichromate distorts the inflammatory reaction induced by turpentine oil injection in male rats. Drug and Chemical Toxicology.2019; ISSN: 0148-0545 (Print) 1525-6014 (Online) Journal homepage: https://www.tandfonline.com/loi/idct20. Scopus CiteScore 2021 - 5,4. Percentile-79, IF-2,597.

4 articles published in journals recommended by the Committee for Control in Education and Science of the Republic of Kazakhstan;

1. M.K. Balabekova, A.N. Nurmukhambetov, R.R. Tukhvatshin, N.N. Ryspekova, **A.N. Tokusheva**, V.V. Trubachev, Zh.E. Aldekeyeva. A modern view on the mechanisms of formation of environmental immunosuppression. Bulletin of KazNMU 2017; pp. 375-380 ISSN 2524-0684 (Print). ISSN 2524-0692 (Online). Citation Index RSCI 2018 – 403 *[The article in Russian]*
2. M.K. Balabekova, R.R. Tukhvatshin, A.N. Nurmukhambetov, N.N. Ryspekova, **A.N. Tokusheva,** V.V. Trubachev, Zh.E. Aldekeyeva. The role of innate immunity in the regulation of inflammation. Bulletin of KazNMU 2017; pp. 375-380 ISSN 2524-0684

(Print). ISSN 2524-0692 (Online). Citation Index RSCI 2018 – 403 *[The article in Russian]*

3. **A.N. Tokusheva,** M.K. Balabekova, Sulev Koks. The influence of polyoxidonium on the activity of B-cells and T-regulatory cells of experimental rats in the dynamics of experimental inflammation. Phthisiopulmonology 2024;.c.150-155. ISSN 2227-1937 (Print). ISSN 2663-1504 (Online) *[The article in Russian]*
4. **A.N. Tokusheva,** M.K. Balabekova, Sulev Koks.Analysis of differential gene expression in the lymph organs of experimental rats. Phthisiopulmonology 2024;.c.156-161. ISSN 2227-1937 (Print). ISSN 2663-1504 (Online) *[The article in Russian]*

Four abstracts published in the materials of international conferences indexed in the Scopus and PubMed databases.

1. M.K. Balabekova, **A.N. Tokusheva,** V.V. Trubachev. Salts of heavy metals cause phenotypic changes of immune competent cells participants and regulators of aseptic inflammation. Molecular biology of the cell, 2017, Vol.28. ASCB an international forum for cell biology. ISSN:1059-1524. https://doi.org/10.1091/mbc.e17-10-0618. Scopus CiteScore 2022 – 6,3. Percentile-92, IF-3,612.

2. Marina K. Balabekova, **A. Tokusheva**, Y. Ostapchuk, N. Abdolla, R. Tuhvatshin. Expansion of His48+CD11b/c+ myeloid cells in rats after vanadium and chromium salts administration. Molecular biology of the cell, 2017, Vol.28. ASCB an international forum for cell biology. ISSN:1059-1524. https://doi.org/10.1091/mbc.e17-10-0618. Scopus CiteScore 2022 – 6,3. Percentile-92, IF-3,612.

3. **Aliya N. Tokusheva,** Marina K. Balabekova, Yekaterina O.Ostapchuk, Nikolay N.Belyaev and Rustam R. Tukhvatshin. Vanadium and chromium mediated impairments in the immunological reactivity of rats with aseptic inflammation. 8th European Immunology Conference 2017.J Clin Cell Immunol 2017, 8:3(Suppl) DOI: 10.4172/2155-9899-C1-037

4. Balabekova M.K., **Tokusheva A.N.,** Trubachev V.V., Belyaev N. N., Aldekeyeva Zh.E., Baratov Z.R., Inkarbek Zh.N., Berdibay Zh.T. Experimental study of the cellularity of lymph organs in rats. International scientific and practical conference "Current scientific research in the modern world", Pereyaslav-Khmelnitsky, Ukraine, 2016. Collection of scientific papers. Issue 10 (18) part 4.p.26-30 ISSN:2524-0986.

One utility model patent:

1. Utility model patent No. 3006. The method of forecasting the chronicity of the inflammatory process in the conditions of intoxication with salts of vanadium and chromium (07.09.2018)

Four articles in international journals (Russian Science Citation Index):

1. Balabekova M.K., Ryspekova N.N., Zhukesheva M.K., **Tokusheva A.N.,** Myrzagulova S.E., Akhmedshina D.A., Trubachev V.V. The dynamics of the course of inflammation caused on the background of metal-induced immunosuppression. 2015; Modern problems of science and education. ISSN 2070-7428.IF RSCI = 1.006. *[The article in Russian]*
2. **Tokusheva, A.N.,** Balabekova, M.K., Myrzagulova, S.E. The reaction of the peripheral blood of rats in response to inflammation caused by intoxication with vanadium and chromium compounds (experiment) 2015; Modern problems of science and education. ISSN 2070-7428.IF RSCI = 1.006. *[The article in Russian]*
3. V. V. Trubachev, M. K. Balabekova, B. Zh. Kasenov, A. M. Karchalova, **A. N. Tokusheva,** D. A. Akhmedshina, Zh. Ryspekova N.N. Experimental studies of the effect of vanadium and chromium compounds on the behavioral responses of rats.2016; Modern problems of science and education. ISSN 2070-7428.IF RSCI = 1.006. *[The article in Russian]*
4. Balabekova M.K., Nurmuchambetov A.N., **Tokusheva A.N.,** Ryspekova N.N., Myrzagulova S.E., Aкhmedshina D.A., Zhukesheva M.K., Trubachev V.V., Yu V.K. Effects of immune modulators at metall induced immunosuppression. International Journal of Applied and Fundamental Research. 2017.

Certificates:

1. Seminar "Strategy and ideology of modern biomedical research focused on genetic epidemiology", 66 hours, September 12-23, 2016; under the guidance of Professor G. Livshits (Israel)
2. Participation in an intensive course on genomic bioinformatics, November 9-11, 2016 (St. Petersburg); under the guidance of Predeus A.

Personal contribution of the PhD student.

The PhD student was directly involved in the development of the idea of the study, the definition of goals and problems, as well as in the design and performance of experiments within the framework of the scientific and technical project of the Ministry of Education and Science of the Republic of Kazakhstan "Molecular and biological features of the course of aseptic inflammation associated with environmental immunosuppression". The PhD student made a significant

contribution to the choice of research methods, data collection and analysis, and interpretation of results.

The candidate actively participated in all stages of the study, including sample preparation, sampling of biological material, analysis of the obtained data, and statistical processing. The author also participated in performing the genome sequencing.

In addition, the candidate took an active part in the promotion of the scientific hypothesis and making conclusions. The author also published the results of the study in relevant scientific journals and conferences.

The structure and volume of the thesis.

The work consists of the introduction, literature review, description of materials and methods, chapter on the experimental research, conclusions, and the list of references (319 references in total).

The thesis is presented on 150 pages containing 30 tables,46 figures and 1 application.