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STUDY OF THE IMMUNOMODULATORY ACTIVITY OF CRYOPRESERVED AND LYOPHILISED HUMAN CORD BLOOD LEUKOCONCENTRATE IN EXPERIMENTAL MODEL OF ATOPIC DERMATITIS

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Abstract. *Atopic dermatitis (AD) is a chronic autoimmune disease characterized by rashes, itching, and dryness of the skin. The drugs of choice for the treatment of AD are steroidal anti-inflammatory drugs, the disadvantage of which is the development of undesirable side effects. Based on this, the search for effective and at the same time safe methods of treating AD is an urgent task of medicine. Based on this, the use of cell and tissue therapy drugs is promising, which have proven themselves as correctors of the immune system, acting on various pathogenetic links of this type of disease.*
The aim of the study was didacated to evaluation of the immunomodulatory activity of lyophilized (lHCBL) and cryopreserved human cord blood leukoconcentrate (cHCBL) in a comparative aspect in the AD model.
The experiments were conducted on 6-month-old Wistar Albino rats. When AD was induced, a focus of inflammation on the back of the rats (3–4 cm^2) was formed by daily rubbing of a 5% alcohol-acetone solution of 2,4-dinitrochlorobenzene (DNCB) for 21 days. The suspensions of lHCBL and cHCBL were administered intraperitoneally at a dose of 0.5 ml of 5 × 10^6 cells one day after the end of DNCB administration. On the 3rd and 7th day after treatment, certain subpopulations of lymphocytes ($CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, $CD4^+CD25^+$), the level of circulating immune complexes, serum concentrations of immunoglobulin A and E, adhesive and phagocytic activity of peritoneal cavity cells were determined.
For AD induced by DNCB, characteristic systemic changes in the immune status are expressed in changes in the indicators of cellular and humoral immunity. Thus, in rats with AD, changes in the cellular immunity in the spleen were detected: namely, a decrease in the number of total T-lymphocytes and their subpopulations ($CD4^+$ and $CD8^+$ cells). Against this background, changes in the humoral and monocyte-phagocytic immunity were noted. The work proved the immunocorrective activity of cHCBL and lHCBL in DNCB-induced AD. In addition, the features of the immunocorrective effect of each of the cord blood preparations, i.e. cHCBL and lHCBL, were noted.
In an experimental model of atopic dermatitis, the therapeutic effect of lHCBL and cHCBL has been proven, namely the ability to correct the state of the impaired immune system of animals. At the same time, a higher immunomodulatory activity of lHCBL was noted compared to cHCBL, which opens up prospects for the use of lHCBL in clinical practice.
Keywords: atopic dermatitis, human cord blood leukoconcentrate cryopreservation, lyophilization, immune system

Atopic dermatitis (AD) is a chronic, recurrent allergic skin disease that causes inflammation, redness, and irritation of the skin. The main factors in the development of AD include both genetic and environmental factors. The pathogenesis of AD is multifactorial, including defects in the cell-mediated immune response, dysfunction of the skin barrier, and changes in the skin microbiota (Boothe, 2024). At the same time, the association of AD with the development of autoimmune genesis disorders is noted (Floca, 2022; De Bruyn Carlier,

2021). The rationale for this concept is the activation of certain parts of the immune system with elements of autoimmune aggression in patients with AD.

An important diagnostic characteristic of mammalian immune system (IS) disorders is the nature of changes in the cellular and humoral components of immunity (Makowska, 2023). AD, in which immune mechanisms are the basis of skin lesions, is no exception. The immune response in AD is characterized by a two-stage inflammatory phase (Baidya, 2025). The “acute phase” is dominated by the T-helper T-type (Th2) immune response, which is characterized by the production of Th2 cytokines such as interleukin (IL)-4 and IL-13. These cytokines activate the production of immunoglobulin E (IgE) and cause IgE-mediated hypersensitivity and eosinophilic inflammation. In the “chronic phase”, the Th2-dominant immune response shifts to a stage of predominance of T1 (Th1) helper cells, characterized by decreased levels of IL-4 and IL-13 and increased levels of IL-5 and IL-12.

T regulatory cells (T-reg) are an important subpopulation of immunocompetent cells (ICCs) that ensure the formation and maintenance of peripheral tolerance to auto- and alloantigens (Goswami 2022). The scientific literature indicates an increase in the number of T-reg cells in the peripheral blood in AD, but with reduced immunosuppressive activity (Inaba, 2023; Reduta, 2023). Due to the ambiguity and sometimes contradictory data on the pathogenetic mechanisms of AD, researchers face an important task of finding effective drugs, including biologically active substances, that help to correct the function of the IS in patients with AD (Alvarenga, 2024). Currently, pharmacy offers a wide range of immunomodulators that have been used in the treatment of AD (Drucker, 2022). These include preparations of animal, plant, and other origin. These drugs do not always demonstrate a high therapeutic effect, and the results of their use in the treatment of AD are sometimes controversial and ambiguous. This fact necessitates the further development and use of new effective and safe immunomodulatory and anti-inflammatory agents to correct the immunocompetence of patients with AD. In this regard, attention should be paid to the multidisciplinary immunocorrective activity of cell therapy drugs. For example, mesenchymal stem cells (MSCs) are used in the treatment of AD (Najera, 2023). Having a high reparative and regenerative potential, these cells have immunomodulatory properties, including tolerogenic ones (Volkova, 2017; Yu, 2023). One of the most promising areas in this field is the development of drugs based on human cord blood (HCB) (Koval, 2021; Koval, 2023; Goltsev, 2020). The therapeutic activity of HCB cells is associated with a unique cellular composition and mechanism of action aimed at normalizing both the state of the IC and the general homeostasis of the body (Wang, 2023; Goltsev, 2023).

A large number of scientific papers indicate the possibility of using HCB cells in the treatment of a wide range of inflammatory processes with elements of autoimmune reactions, namely ischemic stroke, psoriatic arthritis, type I diabetes, Crohn's disease, etc.

The demand for the use of human cord blood leukoconcentrate (HCBL) in clinical practice has determined the need to develop effective methods for their long-term preservation (lyophilization, cryopreservation) (Koval, 2019; Goltsev, 2023; Jahan, 2021). It is important to note that these methods of cryopreservation and lyophilization can act as modifiers of both the structural and functional status of a biological object, in some cases enhancing its therapeutic potential (Goltsev, 2003; Sirous, 2010; Koval, 2019). In view of the above, we conducted a series of experimental studies to determine the effectiveness of the developed new therapeutic approach to the treatment of AD, focusing on the nature of changes in the IS indicators in experimental pathology. This pathology is relevant for humane medicine, and we have worked on it to transfer the proposed method of treating AD to clinical practice.

The aim of the study was to develop a new therapeutic approach to the treatment of AD using lyophilized and cryopreserved human cord blood leukoconcentrate with an emphasis on the evaluation of their immunomodulatory activity.

Methods and materials. The experiments were performed on male rats of 6 months of age weighing 180-200 g in accordance with the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental or Other Scientific Purposes (The European Convention, 1986), as well as in accordance with the Law of Ukraine "On the Protection of Animals from Cruelty" (No. 3447-IV of 21.02.2006). The laboratory animals used in the experimental work were kept in the vivarium of the IPC&C of the National Academy of Sciences of Ukraine.

Leukoconcentrate was obtained from human whole cord blood (HBC) by passive sedimentation in a density gradient with the addition of a 6% polyglucin solution. HCBLs were poured 1 ml into sterile penicillin vials, which were placed on the shelf of the freeze-drying unit UZV-2 (EP IPC&C NAS of Ukraine), and then lyophilized according to the method of Goltsev et al. Samples of lyophilized human cord blood leukoconcentrate (HCBL) were stored at a temperature of 4 ° C. Rehydration of lHCBL was performed by adding 1 ml of saline to the vials for 10 min, stirring gently to prevent foaming. We cryopreserved HCBLs in disposable plastic tubes (Nunc, USA) on the programmatic freezer UOP-6 (EP IPC&C NAS of Ukraine) according to the method of Tsutsaieva et al. The samples of cLECs were stored at 196° C in a low-temperature bank of the IPC&C of the NAS of Ukraine. Thawing of cHCBL was performed in a water bath at 40-41° C. The number of nuclear cells was determined in 1 ml of cHCBL suspension by counting them in a Goryaev chamber. The viability was assessed by flow cytometry (FACS Calibur (BD Bioscience, USA)) using propidium iodide.

AD induction was performed according to the method of the authors (Koval, 2021). This method is based on the use of the hapten 2,4-dinitrochlorobenzene (DNCB), which is able to penetrate the stratum corneum, the upper layer of the skin and covalently modify endogenous proteins, which then acquire the properties of contact allergens. The experimental animals were rubbed daily for 21 days with 0.5 ml of a 5% alcohol-acetone solution of DNCB in previously depilated areas of the back skin (3-4 cm^2).

A total of 35 rats were observed and randomly divided into 5 groups: 1 - intact (control) (n = 7); 2 - AD without treatment (n = 7); 3 - AD + standard treatment (prednisolone ointment) (n = 7); 4 - AD + cHCBL (n = 7); 5 - AD + lHCBL (n = 7). All treatments were started one day after the end of DNCB application.

The immunomodulatory effect of lHCBL was assessed by the degree of recovery of the immune system on days 3 and 7 after treatment.

The number of individual subpopulations of immune cells in the spleen was determined by cytofluorimetry using monoclonal antibodies (MAbs) labeled with FITC (BD, USA) to $CD3^+$ (total T lymphocytes), $CD4^+$ (T helper cells), $CD8^+$ (T suppressors), $CD16^+$ (natural killer cells). To estimate the number of T-reg in the spleen, we used MAT to CD4 and CD25 membrane structures (BD Pharmingen, USA). Immunoglobulins of the same isotypes were used as isotypic controls. Cell fluorescence was determined using a FACS Calibur flow cytometer (BD, USA). All procedures and manipulations with cells were performed in a standard way according to the manufacturer's instructions. Data analysis was performed using WinMDI 2.8 software (J. Trotter, Scripps Research Institute, LA Iolla, USA). At least 10,000 cells were analyzed in each sample.

The study used blood serum sampling, which was carried out in a 10 ml test tube without anticoagulant. Circulating immune complexes (CICs), the concentration of total immunoglobulin A (IgA) and IgE were determined in the obtained sera. To determine the level of IgA in rat serum, the Mancini radial immunodiffusion method was used (Poruchynska, 2021). The concentration of total IgE in the serum of experimental animals was determined by enzyme-linked immunosorbent assay (Anatskyi, 2017). Circulating immune complexes were detected spectrophotometrically (Anatskyi, 2017). The adhesion ability of peritoneal cavity (PC) cells was determined in plastic cups (Spectar, Serbia) at 37° C for 45 min, after which they were gently washed off three times with medium 199. The percentage of adherent cells

(AC) was determined as the difference between the number of cells added and the number of cells in the supernatant (Koval, 2021). The phagocytic activity of AP cells (macrophages) was assessed according to (Koval, 2021, Tatarchuk, 2006).

The results of the study were expressed as median with lower and upper quartiles (Me (LQ; UQ)) and processed using nonparametric statistics using the Statistica 10.0 program (StatSoft, Inc., USA). For multiple comparisons, the Kruskal-Wallis rank analysis of variation was used, and differences were considered statistically significant at $P < 0.05$. In the case of a statistically significant difference, a pairwise comparison of groups was performed using the Mann-Whitney test with the Bonferroni correction when evaluating P values.

Results. The development of immunoinflammatory processes in the mammalian body, regardless of their localization, is manifested by changes in the indicators of both cellular and humoral immunity. The development of AD is no exception to this rule. Indeed, in our studies, in animals with AD, we observed a decrease in the spleen of the total population of T-lymphocytes ($CD3^+$ cells) on day 3 by almost 3 times, on day 7 by 2.7 times (Fig. 1). After treatment of cHCBL and lHCBL animals, a significant increase in the number of $CD3^+$ cells was observed on day 3 compared to that of untreated animals, but only on day 7 did this indicator reach the values of the control group (intact animals). In prednisolone-treated animals, an increase in this indicator relative to untreated animals was observed from day 3, but even on day 7 it was lower than in intact animals.

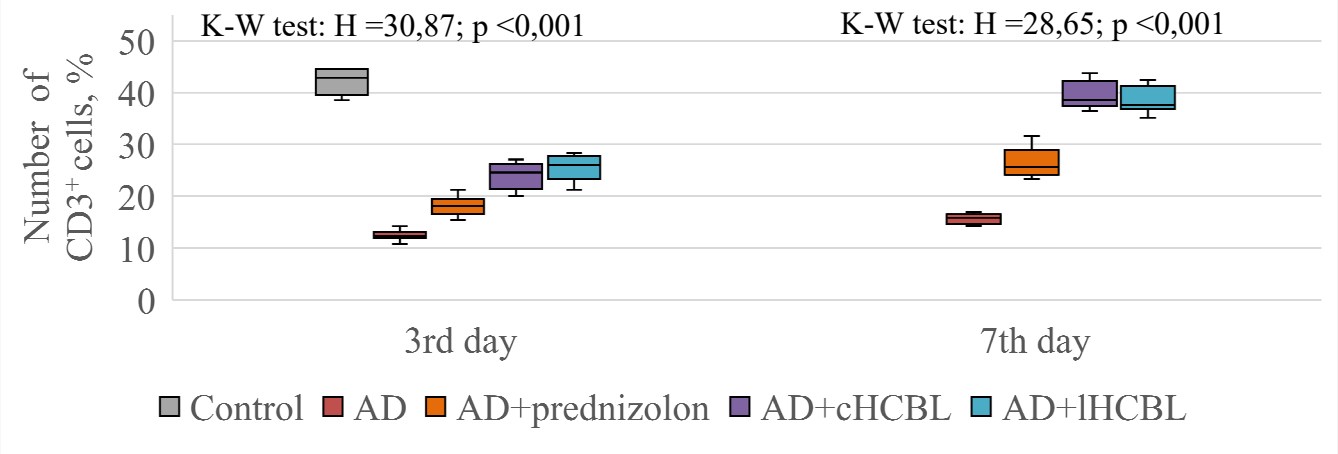

Fig. 1. Dynamics of the total T-lymphocyte population ($CD3^+$ cells) in the spleen of rats with induced atopic dermatitis before and after treatment (n=7).

Notes: * - the difference is statistically significant relative to the control (group 1) ($p < 0.01$); ** - the difference is statistically significant relative to untreated animals with atopic dermatitis (AD, group 2) ($p < 0.01$). The upper and lower borders of the columns correspond to the 75th and 25th percentiles, respectively; the length of the column is the interquartile range; the horizontal line of the column is the median; error bars are the minimum and maximum values.

The number of T-helper cells with $CD4^+$ phenotype in rats with AD decreased by more than 2 times compared with the norm during all study periods.

Evaluation of the dynamics of changes in the content of $CD4^+$ cells in rats of the experimental groups showed a significant increase after treatment in the groups of animals treated with both cryopreserved and lyophilized cells, i.e., cHCBL and lHCBL. At the same time, already on day 3 of the study, a significant increase in this indicator was noted in these groups (up to 24.4% and 25.6%, respectively) (Fig. 2). After 4 days, a further tendency to increase this indicator was noted - its value increased to 30.4 and 28.9%, respectively. In the group of animals treated with prednisolone, this indicator was inferior to that of animals treated with lHCBL, amounting to 20% on day 3 and 22.6% on day 7.

The development of AD in animals was accompanied throughout the study period by a decrease in the number of suppressor/cytotoxic T lymphocytes ($CD8^+$). The level of this

subpopulation of immunocompetent cells (ICC) significantly decreased by day 3, reaching minimum values by day 7 of the study (Fig. 3). Such a significant decrease in the subpopulation of suppressor/cytotoxic T lymphocytes capable of suppressing the activity of syngeneic and allogeneic killers, helper cells, delayed hypersensitivity effectors and lymphocyte blast transformation reaction (LBTR) clearly indicates the development of an inflammatory process in AD animals. In animals with AD with a "burdening" state of IS, the use of lHCBL showed a significant therapeutic effect. Thus, with the use of both cHCBL and lHCBL, starting from the 3rd day, the $CD8^+$ T-lymphocyte content improved sufficiently and did not differ significantly from the values of intact control. It is significant that after prednisolone treatment, despite the higher values compared to untreated animals, this indicator did not reach the values of intact control at all observation periods.

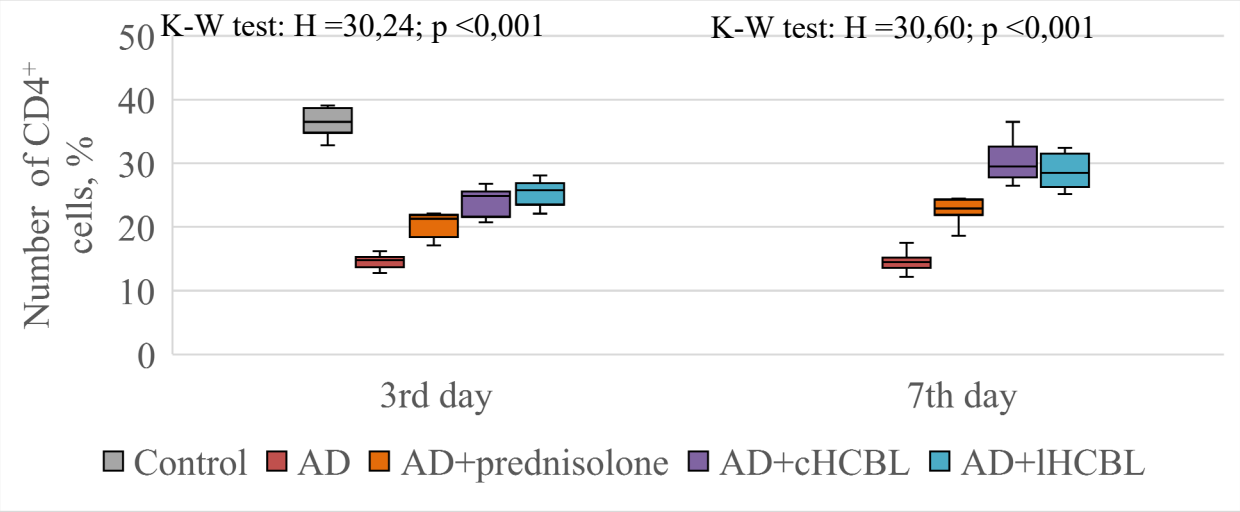

Fig. 2 Dynamics of T-helper ($CD4^+$ cells) content in the spleen of rats with induced atopic dermatitis before and after treatment.

Notes: * - the difference is statistically significant relative to the control (group 1) ($p < 0.01$); ** - the difference is statistically significant relative to untreated animals with atopic dermatitis (AD, group 2) ($p < 0.01$). The upper and lower borders of the columns correspond to the 75th and 25th percentiles, respectively; the length of the column is the interquartile range; the horizontal line of the column is the median; error bars are the minimum and maximum values.

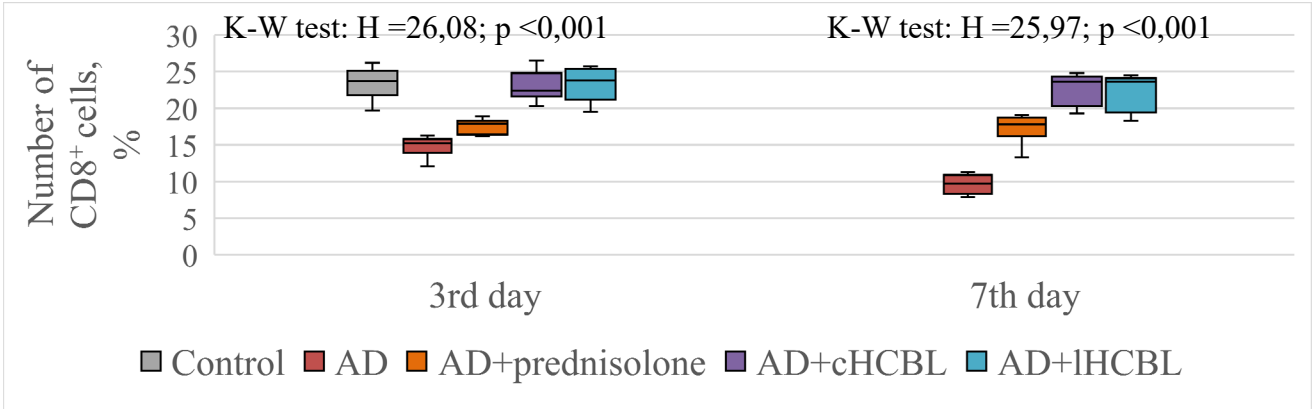

Fig. 3 - Dynamics of the content of suppressor/cytotoxic T-lymphocytes ($CD8^+$ cells) in the spleen of rats with induced atopic dermatitis before and after treatment.

Notes: * - the difference is statistically significant relative to the control (group 1) ($p < 0.01$); ** - the difference is statistically significant relative to untreated animals with atopic dermatitis (AD, group 2) ($p < 0.01$). The upper and lower borders of the columns correspond to the 75th and 25th percentiles, respectively; the length of the column is the interquartile range; the horizontal line of the column is the median; error bars are the minimum and maximum values.

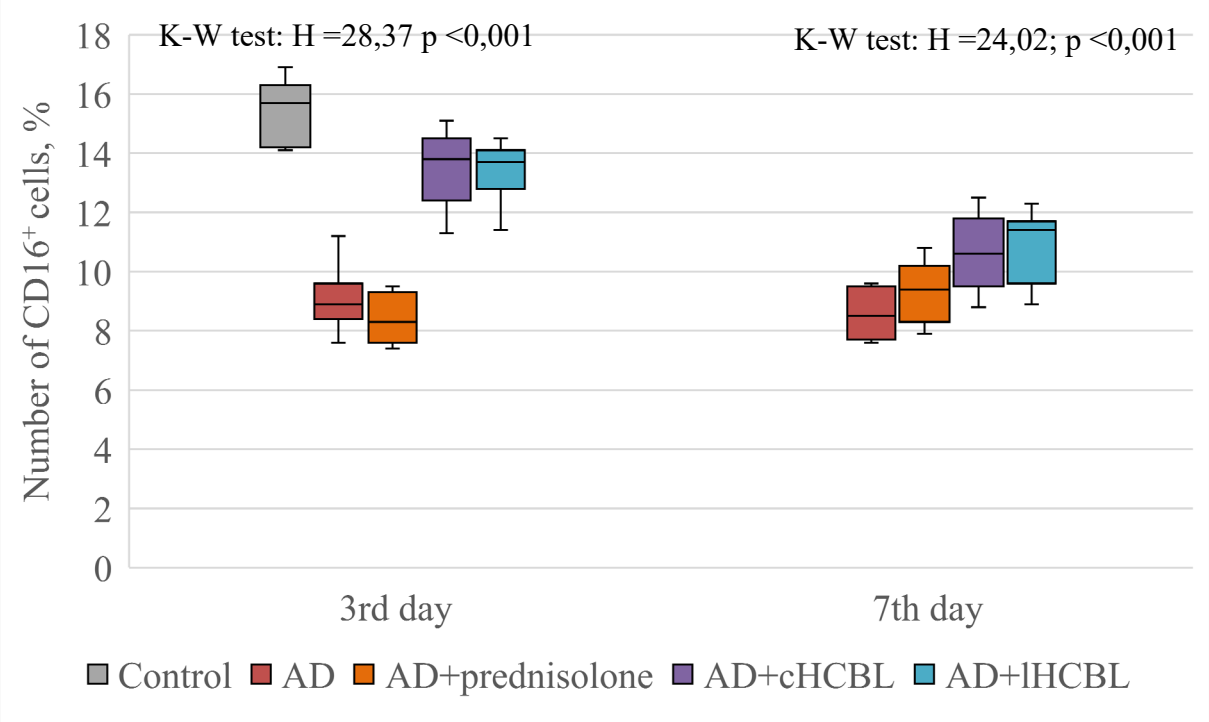

Fig. 4. Dynamics of natural killer cells (CD16⁺ cells) in the spleen of rats with induced atopic dermatitis before and after treatment.

Notes: * **-** the difference is statistically significant relative to the control (group 1) ($p < 0.01$); ** **-** the difference is statistically significant relative to untreated animals with atopic dermatitis (AD, group 2) ($p < 0.01$). The upper and lower borders of the columns correspond to the 75th and 25th percentiles, respectively; the length of the column **is** the interquartile range; the horizontal line of the column is the median; error bars **are** the minimum and maximum values.

As noted above, T-reg cells with the $CD4^+$ $CD25^+$ phenotype, which have immunosuppressive activity, are of utmost importance in the development of autoimmune allergic diseases. It is known that these cells are formed both in the thymus and in the peripheral blood from $CD4^+$ $CD25^-$ cells to provide tolerance to their own antigens in the body of animals. The particular importance of these cells increases with the development of certain pathologies, including autoimmune diseases and inflammatory diseases. The obtained results showed that the content of these cells showed signs of multidirectional changes in the spleen of animals with AD. Thus, the number of T-reg at the beginning of the experiment (day 3) decreased almost 3.5 times compared to the control level and increased 1.3 times above the intact control values on day 7 (Fig. 5). Similar sharp changes in the T-reg content in both directions were observed in the groups of treated animals. It is significant that a similar pattern: a decrease at the beginning of the experiment and an increase in the middle and end of the experiment in the amount of T-reg was recorded in animals treated with prednisolone. After treatment of animals with AD with cHCBL, the T-reg index did not differ significantly from the values in intact controls on day 7. In the animals treated with lHCBL, close values of this indicator to the control occurred only on day 7 of the study. These results indicate that the state of the unique link of the IS, which is T-reg, depends on many factors that change both in the physiological state of the organism, and even more so in the conditions of imbalance of its homeostatic stability, including AD.

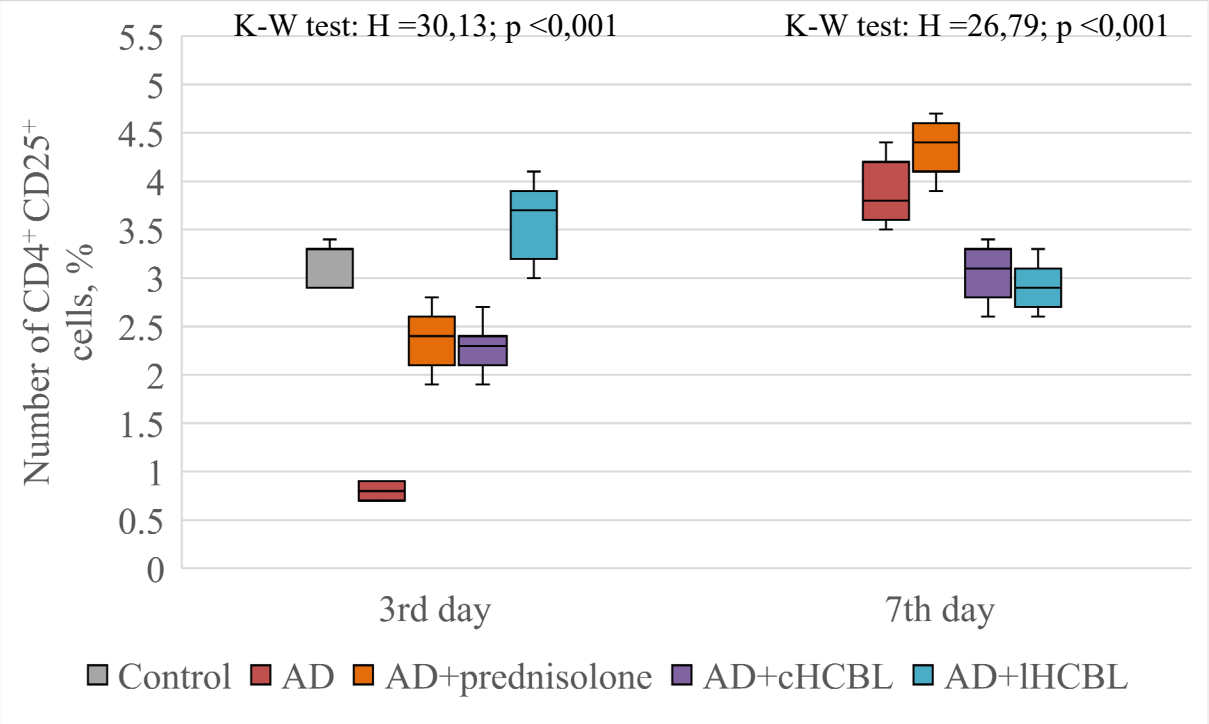

Fig. 5. Dynamics of T-regulatory cells ($CD4^+$ $CD25^+$ cells) in the spleen of rats with induced atopic dermatitis before and after treatment.

Notes: * **-** the difference is statistically significant relative to the control (group 1) ($p < 0.01$); ** **-** the difference is statistically significant relative to untreated animals with atopic dermatitis (AD, group 2) ($p < 0.01$). The upper and lower borders of the columns correspond to the 75th and 25th percentiles, respectively; the length of the column **is** the interquartile range; the horizontal line of the column is the median; error bars **are** the minimum and maximum values.

Circulating immune complexes (CICs) are structures that are formed as an obligatory structural and functional component of the mammalian immune system and consist of different types of immunoglobulins. Changes in their concentration are an indicator of both the degree of activity of the humoral immune system (antibody production) and the activity of cells with phagocytic activity, primarily macrophages.

As a result of the studies, a sufficient increase in the level of CEC in the peripheral blood in rats at all stages of AD development was determined compared with the intact control group: 1.3 times on day 3, 1.4 times on day 7 (Fig. 6). It is significant that only on day 7 after the use of both cHCBL and lHCBL the CEC content reached the normal level. At the same time, after treatment of rats with AD with prednisolone at all stages of the study, the level of CEC remained higher than that of intact animals. It is known that an increase in the concentration of CEC beyond the normal range can be due to at least two reasons. One is the activation of IC cells to hyperproduce antibodies (immunoglobulins of various types) for one reason or another. In addition, a decrease in the number of cells with phagocytic activity (MPS cells) and their absorptive activity. That is, the development of pathology in the form of AD contributes to the development of such a situation with MPS cells. That is, the treatment of animals with AD with HCBL, both in the form of cHCBL and lHCBL, led to an improvement in the functioning of MF of rats with AD.

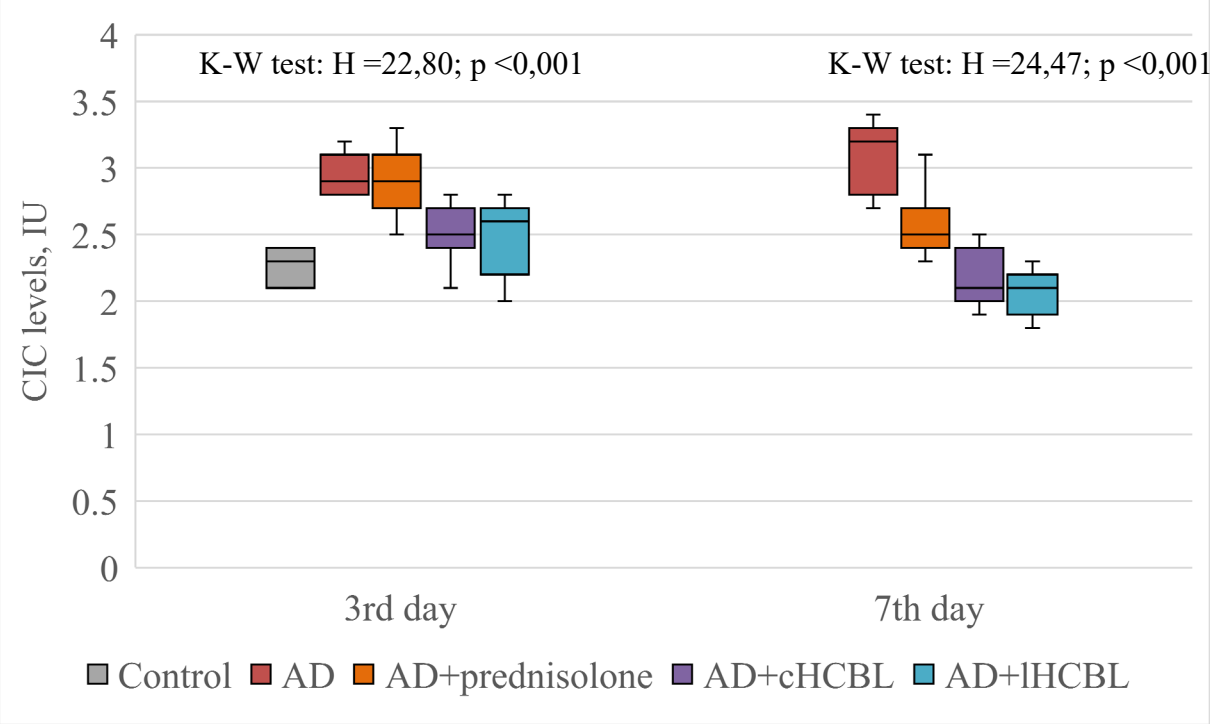

Fig. 6. Dynamics of circulating immune complexes (CIC) in the peripheral blood of rats with induced atopic dermatitis before and after treatment.

Notes: * **-** the difference is statistically significant relative to the control (group 1) ($p < 0.01$); ** **-** the difference is statistically significant relative to untreated animals with atopic dermatitis (AD, group 2) ($p < 0.01$). The upper and lower borders of the columns correspond to the 75th and 25th percentiles, respectively; the length of the column **is** the interquartile range; the horizontal line of the column is the median; error bars **are** the minimum and maximum values.

The role of IgE in the induction and maintenance of allergic diseases, including AD, is known. As can be seen from Fig. 7, the average content of total IgE in rats with AD exceeded ($P < 0.01$) the same indicator in intact rats. This indicator reached its maximum value on day 7 of the experiment and amounted to 186.7 u (Fig. 7). Our results on the increase in the content of total IgE in rats with AD determine the diagnostic value of IgE in the pathogenesis of AD, confirming the allergic etiology of the disease. After treatment of rats with cHCBL and lHCBL, the level of IgE remained increased on day 3 of the study, and on day 7 it decreased to the control level. It is important that despite the positive dynamics, the level of IgE in rats treated with prednisolone even on day 7 remained higher than that of the group of intact animals.

IgA is the most actively produced immunoglobulin in the human body and the second most abundant isotype in the blood serum, second only to IgG. This immunoglobulin, as the main class of antibodies in the secretions that wash these mucous membranes, acts as an important first line of defense.

In untreated rats with AD, the level of IgA decreased more than 5-fold already on day 3 of the pathology, remained at the same level on day 7 (Fig. 8). The IgA levels in rats with AD treated with cHCBL changed unidirectionally, significantly increasing by day 3 of treatment and remaining at the achieved level during the subsequent study period. In the group of rats with AD after treatment with lHCBL, the profile of temporal dynamics during the study was completely different, despite the fact that the level of IgA on days 3 and 7 was 3.6 and 3.4 times higher than in untreated animals, respectively. After prednisolone treatment, the level of IgA was 3 times higher than in untreated animals on days 3 and 7. Thus, as can be seen from Fig. 8, its immunomodulatory potential in relation to this indicator was inferior to that of cHCBL and lHCBL.

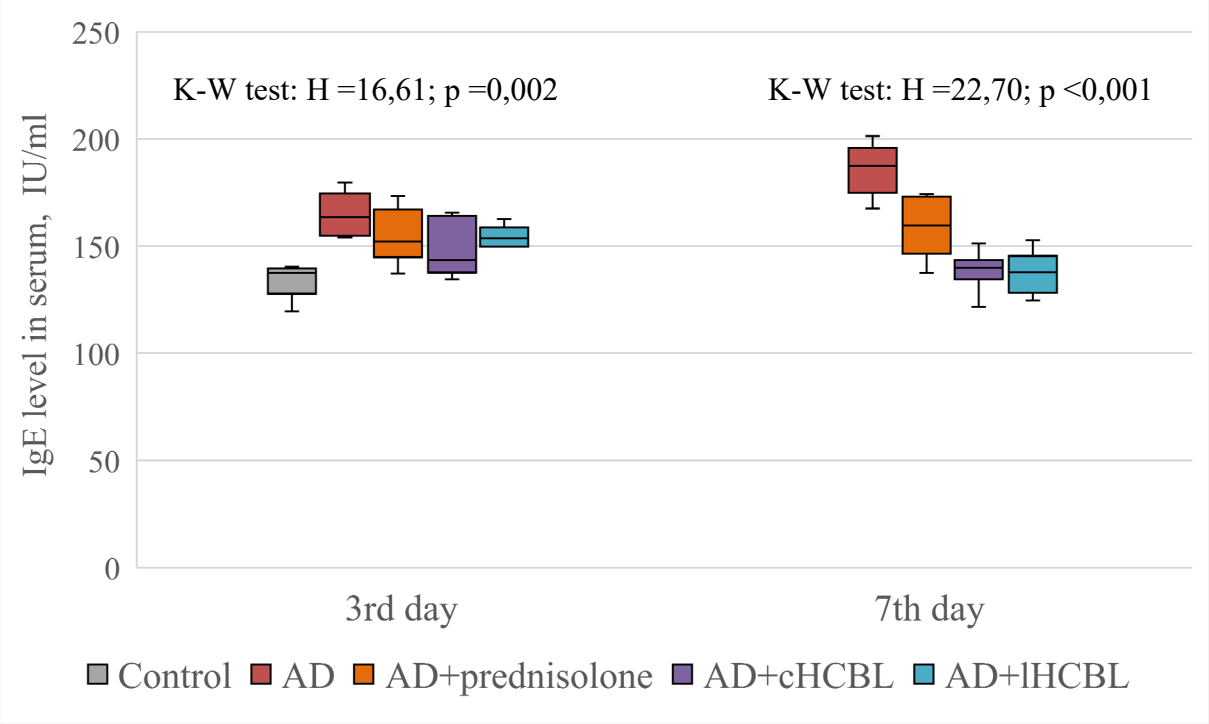

Fig. 7. Dynamics of immunoglobulin E (IgE) content in the blood serum of rats with induced atopic dermatitis before and after treatment

Notes: * **-** the difference is statistically significant relative to the control (group 1) ($p < 0.01$); ** **-** the difference is statistically significant relative to untreated animals with atopic dermatitis (AD, group 2) ($p < 0.01$). The upper and lower borders of the columns correspond to the 75th and 25th percentiles, respectively; the length of the column **is** the interquartile range; the horizontal line of the column is the median; error bars **are** the minimum and maximum values.

It is known that the adhesive potential of animal HCs is a manifestation of the expression of membrane surface receptors such as integrins and selectins. Changes in the body during inflammation can cause a change in the degree of expression of these molecules on the surface of macrophages, thereby modifying their adhesive potential. It should be noted that a change in the adhesive activity of macrophage-phagocyte cells may be a consequence of "affinity modification" as a result of which intracellular signals lead to conformational changes in the extracellular binding sites of adhesion molecules. As can be seen from Fig. 9, the number of adherent cells in the PP in rats with AD increased on day 3 of the experiment and continued to remain at a significantly high level on day 7.

In the group of animals treated with cHCBL and lHCBL, the number of adherent cells was restored to the control level from day 3 of the study and remained at this level until the end of the experiment. After treatment with prednisolone, this indicator, despite a slight decrease in relation to that of untreated animals, remained higher than the control values for all study periods.

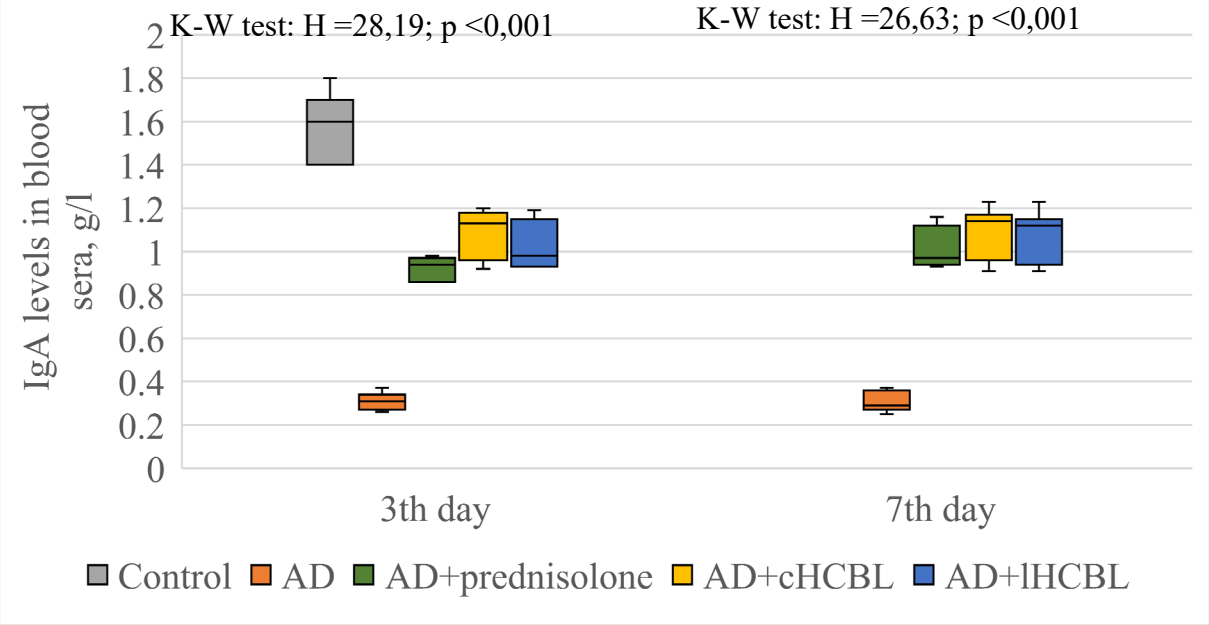

Fig. 8. Dynamics of immunoglobulin A (IgA) content in the blood serum of rats with induced hypertension before and after treatment.

Notes: * **-** the difference is statistically significant relative to the control (group 1) ($p < 0.01$); ** **-** the difference is statistically significant relative to untreated animals with atopic dermatitis (AD, group 2) ($p < 0.01$). The upper and lower borders of the columns correspond to the 75th and 25th percentiles, respectively; the length of the column **is** the interquartile range; the horizontal line of the column is the median; error bars **are** the minimum and maximum values.

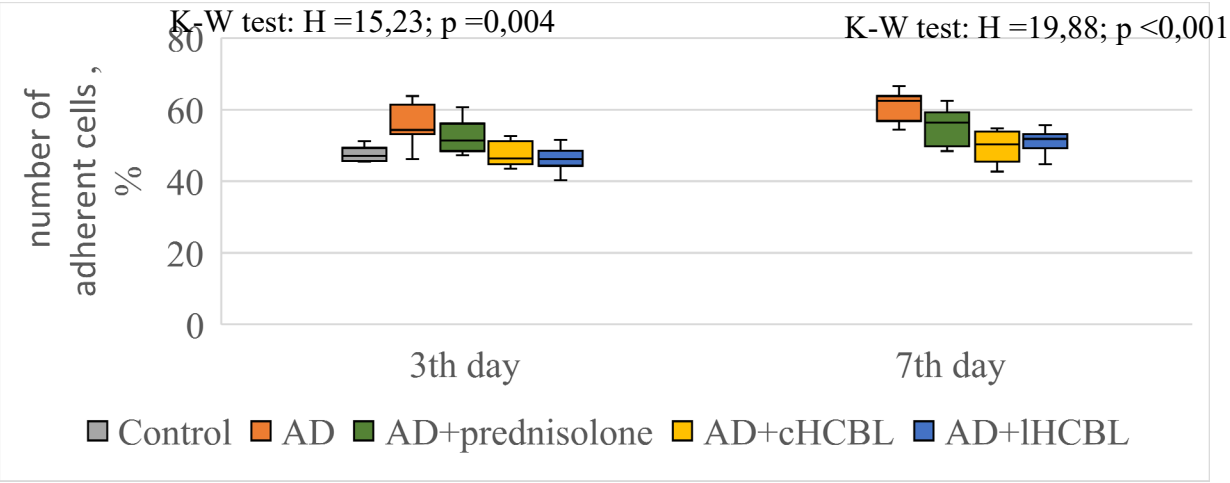

Fig. 9. Dynamics of the number of adherent cells in the peritoneal cavity of rats with induced atopic dermatitis before and after treatment.

Notes: * **-** the difference is statistically significant relative to the control (group 1) ($p < 0.01$); ** **-** the difference is statistically significant relative to untreated animals with atopic dermatitis (AD, group 2) ($p < 0.01$). The upper and lower borders of the columns correspond to the 75th and 25th percentiles, respectively; the length of the column **is** the interquartile range; the horizontal line of the column is the median; error bars **are** the minimum and maximum values.

In a wide range of quantitative and qualitative indicators of the status of the ICC, the phagocyte bactericidal index (PBI) occupies an important place. During the observation period, it was found that in rats with AD, this index decreased sufficiently on days 3 and 7 of the experiment (Fig. 10).

On day 3, in rats treated with cHCBL and lHCBL, the index of PBI was 1.6 times higher than in untreated rats, and on day 7 - 1.8 times higher ($p<0.01$). Despite the increase in PBI

rate in prednisolone-treated rats compared to untreated animals, it remained lower than in the control and in the groups of rats treated with lHCBL at all stages of the study.

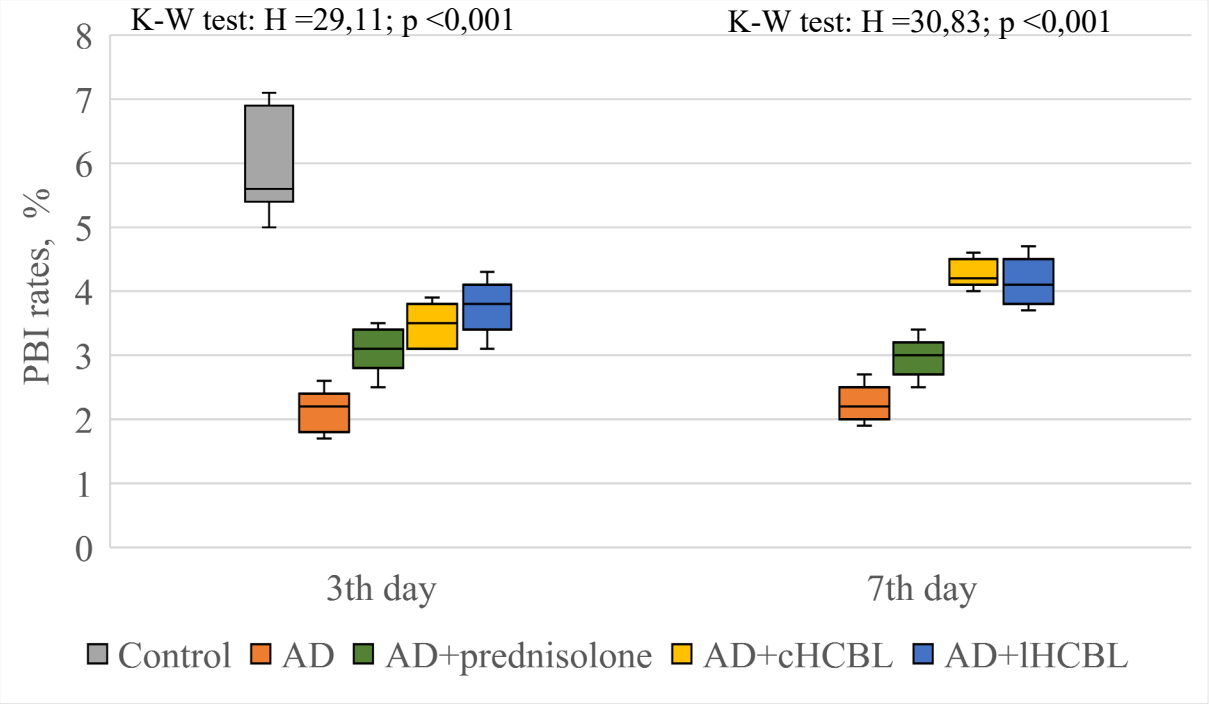

Fig. 10. Dynamics of the phagocyte bactericidal index (PBI) of peritoneal cavity cells of rats with induced atopic dermatitis before and after treatment.

Notes: * **-** the difference is statistically significant relative to the control (group 1) ($p < 0.01$); ** **-** the difference is statistically significant relative to untreated animals with atopic dermatitis (AD, group 2) ($p < 0.01$). The upper and lower borders of the columns correspond to the 75th and 25th percentiles, respectively; the length of the column **is** the interquartile range; the horizontal line of the column is the median; error bars **are** the minimum and maximum values.

Discussion. In the experimental model of DNHB-induced AD, we studied the peculiarities of changes in the parameters of the cellular and humoral immunity and evaluated the possibility of their correction using various forms of cord blood leukoconcentrate. Our data indicate that a characteristic feature of AD is a violation of the cellular immunity, in the form of a decrease in the number of total T-lymphocytes and certain subpopulations of them, natural killer cells. The peculiarities of changes in the content of T-reg cells depending on the stage of AD have been established. Thus, in the early stages (day 3), the number of these cells against the background of AD development was significantly lower than in healthy animals, whereas on day 14 it was significantly higher than in the control. This is in line with clinical data (Inaba, 2023), which noted a higher T-reg content in patients with AD compared to healthy people. Along with this, our studies demonstrated an increased level of IgE, the level of which increased with the development of AD, which coincides with the clinical data of other authors (Atwal, 2023; Pellefigues C. 2020).

One of the main tasks of therapeutic approaches to the treatment of AD is to restore the functional state of the IS impaired by this pathology. Such an approach can be based on the use of human cord blood leukoconcentrate (HCBL), which is essentially a part of the fetal blood. Cord blood and its components are widely used in modern medical and biotechnological programs due to their unique multifunctional properties (Wang, 2023).

The paper provides a clear evidence base for the ability of HCBLs to act as a therapeutic agent for experimental AD in animals, accompanied by the manifestation of immunomodulatory properties against the background of restoration and approximation of the indicators of the IS of rats with AD to the values of healthy animals. The clinical efficacy of cord blood cells has

been shown in many inflammatory and autoimmune diseases (Coutts M, 2017; He B, 2015; Berglund S; 2017; Zhang, 2018; Qi, 2020).

It is well known that both the degree of manifestation of a particular allergic disease and the manifestation of the therapeutic effect of various drugs in relation to it depend on the timing of the identification of these indicators (Mochulska, 2015). Earlier, we showed the ability of HCBLs to reduce the manifestation of dysregulation of the state of the IS in AD on day 14 of the development of this pathology (Koval, 2021). In the present study, we focused on the effect of HCBL on the indicators of IS in animals with AD at the early stages of this pathology (days 3 and 7 of AD development). We consider the immunocorrective effect of cryopreserved and lyophilized HCBLs in rats with AD as evidence of a pathogenetically based and targeted treatment regimen for the pathology in the experimental setting, which is based on the dysfunction of the IS. The ability of these cells to produce certain regulatory mediators in the treatment of AD is multifaceted and is determined by the level of expansion of the immunoinflammatory process in the body. The diversity of mechanisms of action of HCBL on different parts of the IS involved in the pathogenesis of AD ensured the correction and harmonization of the immune response of cellular and humoral immunity. Our work has shown that this kind and level of activity is characteristic of HCBL, since after the use of HC the immunomodulatory effect was less pronounced. At the same time, the nature of the effect of cHCBL and lHCBL on the indicators of IS depended on the stage of the experimental pathology. It should also be noted that the degree and nature of the effect of cryopreservation factors on a biological object depend on the conditions of the freezing-thawing process (Ostankov 2008).

In this case, the results of a comparative assessment of the effect of lyophilized and cryopreserved HCBL are quite interesting. As can be seen from our data, a more pronounced positive effect on the Treg content was observed in animals treated with lHCBL. Moreover, this effect was observed only on day 3 of observation. Obviously, the differences in the immunomodulatory effect of lyophilized material (lHCBL) on the Treg content in the spleen compared to cryopreserved material (cHCBL) may be due to a number of reasons. Firstly, a special change in the quantitative and qualitative composition of HCBLs after lyophilization. It has been established that some lyophilization regimens, including the one used in this study, can have a selective effect on certain subpopulations of cord blood cells, increasing the content of T-reg cells (Koval, 2019). Possessing a high proliferative activity, they can increase the pool of T-reg cells in the spleen when administered to animals with AD using lyophilized material. The different ability of cryopreservation and lyophilization to change the profile of mediators produced by HCBLs may determine their different therapeutic potential.

Conclusions. The results of the study showed that the development of AD induced by DNCB throughout the experiment is accompanied by a violation of the cellular immunity, namely, a decrease in the number of CD3+, CD4+, CD8+, CD16+ cells in the spleen. At the same time, in the early stages (3 days), a decrease in the content of CD4+CD25+ cells (Treg) is observed. Against this background, an increase in the production of IgE and CEC with an antiphasic increase in the concentration of IgA was recorded. Significant in the context of AD development is the established fact of imbalance of the MPS, namely, an increase in the number of adhesive cells against the background of a decrease in their phagocytic activity. The fact of increased activity of T-reg cells formation in the spleen on day 7 with no potential to resist the manifestation of pathology signs (AD) is noteworthy.

Minimization of signs of the degree of inflammatory process manifestation with the use of both forms of cord blood leukoconcentrates in animals with AD demonstrates the multidirectionality of both the mechanism and targeting of targets for the manifestation of the immunocorrective activity of HCBL at different levels of the body organization, which leads to the normalization of immunity. It is important to conclude that the anti-inflammatory targeting profile for both forms of HCBL is significantly greater than that of HC.

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ДОСЛІДЖЕННЯ ІМУНОКОРЕГУЮЧОЇ АКТИВНОСТІ КРІОКОНСЕРВОВАНОГО ТА ЛІОФІЛІЗОВАНОГО ЛЕЙКОКОНЦЕНТРАТУ КОРДОВОЇ КРОВІ ЛЮДИНИ В ЕКСПЕРИМЕНТАЛЬНІЙ МОДЕЛІ АТОПІЧНОГО ДЕРМАТИТУ

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*Резюме**. Атопічний дерматит (АД) є хронічним захворюваням аутоімунного генезу, що характеризується висипаннями, свербінням, сухістю шкіри. Препаратами вибору лікування АД є стероїдні протизапальні засоби, недоліком яких є розвиток небажаних побічних ефектів. Виходячи з цього пошук ефективних і в одночас безпечних методів лікування АД є актуальним завданням медицини. Перспективним виходячи з цього є застосування препаратів клітинної і тканинної терапії, зарекомендували себе як коректори стану імунної системи, що діють на різні патогенетичні ланки такого типу захворювання.*

Мета дослідження полягала у оцінці в порівняльному аспекті імунокорегуючої активності ліофілізованого (лЛККЛ) і кріоконсервованого лейкоконцентрату кордової крові людини (кЛККЛ) на моделі АД.

Експерименти були проведені на 6-місячних щурах лінії Вістар. При індукції АД вогнище запалення на ділянці спини щурів (3–4 см^2) формували щоденним втиранням 5 %-ного спиртово-ацетонового розчину 2,4-динітрохлорбензолу (ДНХБ) протягом 21 доби. Суспензію кЛККЛ і лЛККЛ вводили внутрішньочеревинно по 0,5 мл у дозі 5-10^6 клітин через добу після закінчення застосування ДНХБ. На 3 та 7 добу після лікування були визначені певні субпопуляції лімфоцитів (CD3$^+$, CD4$^+$, CD8$^+$, CD16$^+$, CD4$^+$CD25$^+$), рівень

циркулюючих імунних комплексів, концентрації імуноглобулінів А та Е у сироватці крові, адгезивна і фагоцитарна активність клітин перитонеальної порожнини.
Для АД, індукованого ДНХБ, характерні системні зміни імунного статусу, що маніфестують ся змінами показників клітинного і гуморального імунітету. Так, у щурів з АД у щурів виявлено зміни клітинної ланки імунітету у селезінці: а саме зниження кількості загальних Т-лімфоцитів і їх субпопуляцій (клітин CD4⁺ і CD8⁺). На цьому тлі відзначені зміни гуморальної та моноцитарно-фагоцитарної ланки імунітету. У роботі доведена імунокорегуюча активність кЛККЛ і лЛККЛ при ДНХБ індукованому АД. Крім того відзначені особливості імунокорегуючого ефекту кожного з препаратів кордової крові, тобто кЛККЛ і лЛККЛ
В експериментальніймоделі атопічного дерматиту доведено лікувальний ефект лЛККЛ та кЛККЛ, а саме здатність корегувати стан порушеної імунної системи тварин. При цьому, відмічена більш висока імуномодулююча активність лЛККЛ в порівнянні з кЛККЛ, що відкриває перспективи застосування лЛККЛ у клінічній практиці.
Ключові слова: атопічний дерматит, лейкоконцентрат кордової крові людини, кріоконсервування, ліофілізація, імунна система.

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