

COMPARATIVE EVALUATION OF IMMUNOLOGICAL PARAMETERS OF LABORATORY ANIMALS WITH THYMUS AUTOIMPLANTATION IN THE DYNAMICS OF OBSERVATION

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ABSTRACT

The paper presents the results of a comprehensive immunomorphological study during autoimplantation of the thymus. An experimental study was carried out on rabbits of the Chinchilla breed by modeling thymectomy followed by autoimplantation. Correlation relationships of clinical and immunological parameters in the experiment were revealed.

To date, congenital heart defects (CHD) in children remain one of the causes of disability and mortality among children. The most common among children are ventricular septal defect in 28.3% of cases, atrial septal defect in 10.3%, pulmonary artery stenosis in 9.8%, tetralogy of Fallot in 9.7%, aortic stenosis in 7.1%, coarctation aorta in 5.1% and transposition of the great vessels in 4.9% of cases. There are also hypoplastic tricuspid valve syndrome, open ductus arteriosus, complete abnormal venous return [1,9,10].

The issue of adaptive capabilities, tactics of long-term observation and rehabilitation of children with congenital heart defects is debatable [10]. CHD in children is complicated by a decrease in the rate of physical development, which requires constant monitoring [7,8]. Currently, the correction of the most common congenital heart defects in children can be carried out by two main methods: open surgery and endovascular procedures [3,9,11,17].

Lymphocytes are responsible for the maintenance of genetic homeostasis in the body through the development of specific immunity and the implementation of immune surveillance. The primary differentiation of null lymphocytes occurs in the main organs of immunity, where these cells turn into T- and B-lymphocytes. T-lymphocytes are responsible for cellular immunity, and B-lymphocytes for humoral immunity, by producing antibodies [1,2,4,15,16].

Approximately 75% of the total number of lymphocytes circulating in the blood and lymph are T-lymphocytes, while the proportion of B-lymphocytes is approximately 15%, and 10% are cells that do not belong to any of the above groups [2,12,14].



The degree of resistance of animals to diseases is determined by the level of natural resistance, which is directly dependent on age, season, conditions of feeding and keeping [1,2,13].

One of the main cells of adaptive immunity are T-lymphocytes. The population of T-lymphocytes have heterogeneous properties. Most of them are formed in the thymus. The thymus gland, as the central organ of immunity, conducts antigen-independent differentiation of T cells. It should be noted that the thymus gland is extremely sensitive to various exogenous and endogenous influences, especially in childhood. The impact of the above factors will lead to a violation of the structure of the thymus gland and, as a result, its function, which causes dysfunction of the immune system as a whole [2,3,6,11].

Purpose of the study: to also evaluate the correlation between the indicators of immunity and physical development in laboratory animals with autoimplantation in the dynamics of the postoperative period.

Materials and methods:

Experimental studies were carried out in two stages: the first stage was the modeling of thymectomy in 1-2 groups in compliance with all the rules of asepsis and antisepsis. Under general anesthesia, Calypsol solution was intramuscularly injected at the rate of 6-8 mg/kg. After anesthesia, the rabbits were fixed on the operating table without stretching. Previously, depilation and 3-time treatment with an antiseptic (1% alcohol solution of iodine) of the surgical field was performed.

Further, along the anterior midline, a skin incision was made with dissection of the fascia and periosteum of the sternum. Access to the fatty tissue of the mediastinum was created by dividing the sternal part of the diaphragm and the opposite surface of the sternum. The sternum was dissected in stages, all over. In order to stop bleeding from the edges of the sternum, surgical (sterile) wax was rubbed. After the end of the sternotomy, the thymus was found. Next, a thymectomy was performed, the thymus was removed bluntly. After removal of the thymus, autoimplantation of 1/3 of the organ was performed on the pericardium in the projection of the right atrium, adjacent to fatty tissue for better engraftment and vascularization of the organ [63; With. fourteen]. The autoimplant was fixed with 5.0 Vicryl suture. Hemostasis, the edges of the sternum were closed with titanium sutures.

When working with laboratory animals, all the rules of biological safety and ethical principles of working with laboratory animals were observed

Immunological studies, were held at the Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan (Tashkent). T-lymphocytes were determined by ELISA: young thymocytes (CD3+/-, CD4+/-, CD8+/-)-participating in antigen-independent differentiation of T-cells, natural killers (EK-CD16+/-), lymphocytes of early (CD25+/-) and late activation (CD95+/-).

To study the relationship of physical growth and development with immunity indicators, we conducted a correlation analysis based on the data obtained during the pilot study. Communication criteria were assessed on the Chaddock scale, with:

- $0.1 < r < 0.3$: weak connection;
- $0.3 < r < 0.5$: moderate association;
- $0.5 < r < 0.7$: significant relationship;



- $0.7 < r < 0.9$: high association;
- $0.9 < r < 1$: very high association.

Results and discussion:

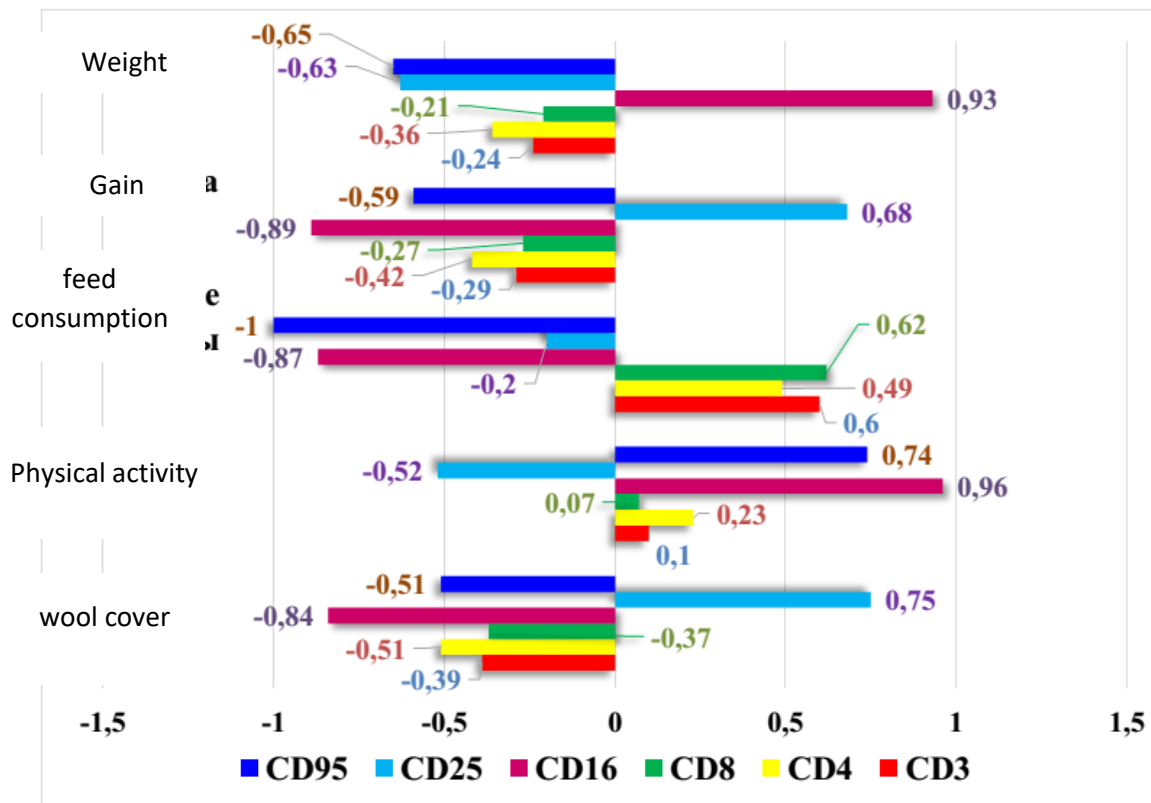
The condition of rabbits with autoimplantation was monitored, weight, volume of food consumed and coat density were measured weekly. At the same time, to study the state of the thymus, an analysis of cellular immunity was performed.

For comparison with the control group and rabbits of the 2nd group (with thymectomy), a correlation analysis of immunity parameters with indicators of physical growth and development of rabbits of the 1st group was carried out.

In contrast to the data obtained from the correlation analysis of the 2nd group, in rabbits of the 1st group, a moderate negative relationship was revealed between the weight index and CD3+ lymphocytes - $r=-0.39$ and a noticeable positive relationship between motor activity and CD3+ lymphocytes - $r= 0.60$.

Noticeable negative relationships were also established between CD4+-lymphocytes and weight ($r=0.51$), volume of feed consumption ($r=-0.42$) and coat density ($r=-0.364$). And the motor activity of rabbits has a noticeable positive relationship with CD4+ -lymphocytes ($r=0.49$). At the same time, an interesting fact was obtained confirming the positive dynamics of the state after autoimplantation: weekly weight gain has a weak positive relationship with the level of CD3+- and CD4+-lymphocytes, there is no connection with suppressors (CD8+-lymphocytes). At the same time, weak negative relationships were found between CD8+-lymphocytes and the volume of feed consumption - $r=-0.27$ and wool density $r=-0.208$. Motor activity in rabbits of the 1st group has a high positive relationship with CD8+-lymphocytes - $r=0.62$.

CD16+-lymphocytes responsible for transplantation immunity showed a high positive relationship with the weekly weight gain of rabbits ($r=0.96$) and wool density ($r=0.92$), as well as a high negative relationship with weight indicators ($r=-0.84$), physical activity ($r=-0.87$) and feed consumption ($r=-0.89$). The relationships obtained make it possible to predict graft healing based on weekly weight gain and coat density in rabbits. At the same time, a decrease in motor activity and food intake confirms the unfavorable outcome of thymus autoimplantation (Fig. 1.).



Rice. 1. Correlation relationship between immunity and physical development indicators in rabbits with autoimplantation

CD25⁺ cells, as markers of early activation, show the process of healing or graft rejection. The study found a high positive relationship of CD25⁺-cells with weight and feed intake in rabbits with autoimplantation (group 1), $r=0.75$ and $r=0.68$, respectively. At the same time, a peculiarity in the development of the early stage of the inflammatory process was revealed, which affects the condition of the coat and weight gain in rabbits, which confirms the presence of a negative high correlation between CD25⁺-lymphocytes and coat density $r=-0.634$, between CD25⁺-lymphocytes and weekly weight gain $r=-0.52$.

CD95⁺ cells are important in controlling the functioning of the immune system and show a process of apoptosis-cell death. A decrease in their concentration in the blood makes it possible to predict the recurrence or chronicity of the inflammatory process. And an increase in the level of CD95⁺-cells confirms the development of sepsis and viral diseases.

In the course of the correlation analysis, a high positive relationship of CD95⁺ cells with weekly weight gain was established $r=0.74$. At the same time, a noticeable negative relationship was found between CD95⁺-cells and feed intake $r=-0.59$ and the density of rabbit hair $r=-0.65$. The data obtained prove the possibility of predicting the outcome of autoimplantation according to the condition of the coat and food intake in rabbits of the 1st group.

Therefore, it was possible to predict the development of sepsis during autoimplantation based on monitoring the condition of the coat and feed intake. Motor activity at the same time shows no difference in the development of the septic process between the two indicators.

In this way, correlation analysis makes it possible to predict the outcome of autoimplantation and the probability of rejection of the transplanted piece of thymus in



rabbits. The established relationships between the parameters of cellular immunity and the indicators of physical growth and development of rabbits make it possible to predict the healing of the graft based on weekly weight gain and coat density. At the same time, a decrease in motor activity and food intake confirms the unfavorable outcome of thymus autoimplantation.

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