

# **THE COMPARATIVE ANALYSIS OF EFFICIENCY OF USING THE “ROSETTE” METHOD AND THE METHOD OF IMMUNOTYPING BY MONOCLONAL ANTIBODIES TO CONTROL THE STATE OF THE IMMUNE SYSTEM OF THE ALMOST HEALTHY DOGS AND THE DOGS WITH DERMATITIS**

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The article presents the data of comparative analysis for determining the number of T-lymphocytes using the “rosette” method and the method of immunotyping by monoclonal antibodies. It's proved that almost healthy dogs don't have significant differences in the relative number of T-lymphocytes in the peripheral blood.

***The keywords: dogs, immunity, T-lymphocyte, T-helper, T- suppressor, immunophenotyping.***

It is known that the most informative tests that characterize a potential ability of the immune system to an adequate immune response is an analysis of the number of T and B lymphocytes, activated T-lymphocytes in the peripheral blood of patients. The discovery of the hybridoma technology for monoclonal antibodies and the introduction into practice of clinical diagnostic laboratories of automatic analysis based on quantitative flow cytometry flavored further displacement of “rosette” tests. The modification of the test enables to single out different "useful" variants from E-RFC such as sensitized T-lymphocytes to different antigens of organs and tissues. From the position that the method of plaque is still a widely used test in the practice of immunological laboratories, we thought it necessary to conduct a comparative analysis of determining the number of T, T lymphocytes activated by the results of two tests: the spontaneous rosette (E-RFC) and CD- typing (population of CD3 + , CD4 +, CD8 +) in order to assess the most informative method for monitoring the state of the immune system in healthy and diseased dogs dermatitis.

**Materials and methods.** Work was written at the Department of Physiology, Biochemistry and Microbiology of the Odessa State Agrarian University in collaboration with the Laboratory of Immunology of the Institute of Eye Diseases by VP Filatov. The material for the study was previously stabilized peripheral blood of dogs, aged 1-9 years ( $n = 38$ ) which had the same housing and feeding conditions. Animals are divided into three groups: Group 1 ( $n = 14$ ) apparently healthy dogs aged 1-5 years; Group 2 ( $n = 11$ ) apparently healthy dogs aged 6-9 years; 3rd group ( $n = 13$ ) dogs with dermatitis. Blood was collected in the morning on an empty stomach from the ulnar vein in a test tube with EDTA. The evaluation of lymphocyte population in rosette tests and immunophenotyping was taken from animals. An absolute and relative content of lymphocytes and their subpopulations in the reaction of rosette with sheep erythrocytes was determined in blood (E-tf.r.-RFL, e-tf.ch.-RFL) [1]. Immunophenotype was estimated according to the recommendations of the company AbD Serotec (UK) using reaction immunofluorescence. These data are statistically processed using the Student's t-test.

**The results of research.** As the results of studies showed in the blood of practically healthy dogs (Group 1) circulates  $62,0 \pm 4,0\%$  - CD3 + lymphocytes and virtually the same amount of  $61,33 \pm 15,14\%$  - E-RFC. The results of immunological studies in dogs after 6 years have shown that the relative amount of T-lymphocyte in rosette formation reaction 2% more than the reaction immunotyping T cells with monoclonal antibodies. Dogs with dermatitis have 7.25% bigger difference in the relative amount of T-lymphocytes in response to rosette than the number of these cells in the reactions of monoclonal antibodies with CD3 +. Quantitative characterization of subpopulations of T-helper cells (CD4 +) activity shows a significant increase in their relative level with respect to the index of old dogs with dermatitis period according to the results of the reaction of rosette. The results obtained with help of rosette reaction about content of T-cells with suppressive (CD8 +) activity indicate that low levels of these cells in the reaction of immunophenotyping.

**Conclusion.** Based on the analysis of studies determining the number of T-lymphocytes using rosette method and immunotypology method with help of

monoclonal antibodies it is discovered that practically healthy dogs aged until 6 years relative number of these cells is within 60% with age the after the age of 6 years content of T- lymphocytes in the peripheral blood decreases.

The data obtained can be used in the interpretation of immune diagrams parameters in the clinic veterinary practice of pets assessing immune physiological reactivity of the organism and carrying out of treatment.