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Research Article

Quantitative and Immunohistochemical analysis of lymphoid cells of swine Immunized with Escherichia coli bacteria

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ABSTRACT

In this study we have demonstrated lymphoid cells in the small intestine, spleen and mesenteric lymph node (MLN) of the 4 weeks old pigs, crossbreeds between swedish Landras, Hypor and Seghers, after immunization with nonenterotoxigenic (non – ETEC) *Escherichia coli* strain.

Using the immunohistochemical ABC method, with the monoclonal antibodies MIL 13, specific for the CD45RA marker and MIL5, specific for the CD45RC marker, we showed the histological distribution of the CD45RA, and CD45RC lymphoid cells in MLN and small intestine. In the MLN of immunized pigs CD45RA cells are distributed in large numbers in borth cortex and paracortex, in the small intestine we found accummulations in the lamina propria and individual cells in the epithel. CD45RC cells are fev in the small intestine, in the paracortex of the MLN they are mumerous, and only few in the cortex and the sinuses.

Quantitative data for MLN, spleen and the Peyer's patches of ileum were obtained from the flow cytometer. CD45RA marker was found in the larger percentages in the experimental group than in the control group in all of the above organs. Also, CD45RC marker was found in larger percentages in MLN and sleen, while in the Peyer's patches of the experimental group it was in smaller percentage when compared to the control group.

These results indicate icrease in the percentage of the lymphoid cells in all of the mentioned organs of immunized animals, and therefore possible immunogenicity of the non – ETEC *E. coli* strain.

Keywords: Lymphoid cells, Swine, Immunization and Escherichia coli.

1. INTRODUCTION

Cells that are participating in the immune system, leukocytes, mobile units present in the body's defense system [5, 7]. Some of them are formed in the bone marrow (granulocytes, monocytes and small number of lymphocytes), a part of lymphatic tissue (lymphocytes and plasma cells). In normal blood there are 6 different types of leukocytes [8, 13, 15], which are: polymorphonuclear neutrophils, eosinophils, polymorphonuclear, polymorphonuclear basophils, monocytes, lymphocytes, plasma cells and more platelets that are part of the 7th kind of leukocytes which are found in marrow bone-megakariocyte. All of its three kinds of polymorphonuclear leukocytes have a granular structure for what are called granulocyte. Granulocytes and monocytes protect the body from disease causes phagocytosis, ie with their ingestion. T and B Establish gland lymph organs, thymus and bone marrow. From here lymphocytes pass in peripheral lymphoid organs: spleen, lymph nodes and Peyer's platches the ileum where it ends their differentiation. Developed two similar immune systems, cellular immunity which react with microorganisms immunocompetent cells, foreign cells (tumor or transplantation). For this immunity responsible are T lymphocytes. The other type is the humoral immunity, which focuses on blood

glycoproteins, which are called antibodies that inactivate or eliminate foreign agents [3, 4]. Antibodies (immunoglobulins) are blood plasma glycoprotein that relate to antigenic determinants which encourage their creation and those I share in some classes (IgG, IgA, IgM, IgE, IgD) [1, 2, 10, 16]. Some cells suckle IL similar to hormones, which control the activity of cells that are part of the immune reaction [11, 14].

2. MATERIALS AND METHODS

In evidence are dealt 7 healthy pigs Swedish Hyporit landrace crucifixion and Segherses age of 4 weeks. The pigs were divided into two groups: 4 in the experimental group, who were immunized with 10 cfu / ac ml. F4 of type Abbotstown in 60 ml triptikaz Bujon strain (TSB) I had with 1.2% NaHCO3, 3 animals were equally treat with 60 ml TBS add 1.2% NaHCO3 . Animals killed 5, 6 or 7-en days after immunization. Was injected intravenously 0.3 ml / kg T61. Imunohistochemical methods and painting ABC - imunohistochemical methods using detected with specific antibodies components that carry antigenic determinants. For the presentation of the reaction between the antigen and antibodies used citochemical reagents. One of the methods is very precise in Histochemistry use of avidin-biotin complex, which is used in all research areas of molecular biology, cell, histology, immunology, clinical biology and pathology. Marking with primare- antibodies of mice used monoclonal antibodies.

3. RESULTS

With this research of imunohistochemical through ABC method, is demonstrated localization and distribution of CD 45 RA, CD 45 RC lymph cells in lymph tissue in the digestive system pigs drowned 6 and 7 days after immunization. In the ileum of pigs tested with monoclonal antibodies which marks CD 45 RC, leukocyte cell population appears very rarely CD 45 RC + cells in the intestinal epithelium. In jejunum rarely I find CD 45 RC cells. In the paracortex NLM find large numbers of cells CD 545 RC, and in the region of cortex are rare and lonely, but are also present in the sinuses of the MLC. During acquired quantitative research shows that participation of citometric flow cell CD 45 RA is greater in the ilium Peyer's platches in the group of experimental animals compared with Peyer plates of the group of control animals, the presence of cells CD 45 RC is smaller Peyer's platches animals in the experiment compared to the control, (Fig.1).

4. DISCUSSION

The surface of the mucous membranes of digestive system is under the influence of a large number of different antigens, including harmful substances without food, but also large potential pathogens [9, 16, 17]. However these differences mechanism is not yet completely clarified, control Conduct for antigens and protection system is achieved by a combination of specific and non-specific protective organisms. Research so far showed that the epithelium of the intestine of swine represents only immune microenvironment [12]. Considerable number of cells present in the epithelium, Peyer's platches, jejunum and ileum are participating in the surveillance of the immune system [6]. Results show increasing presence of CD 45 RC and speeding subpopulation differentiation of T helper.

5. CONCLUSION

Based on what was said above, we can conclude that:

1. The tissue distribution of T lymphocytes and lymphoid cells, mijeloide CD 45 and CD 45 RC RA, in mesenteric lymph node (NLM) pigs immunized animal shows of absorption quality issues raised, while the number in lamini propriji the jejunumit and peyer plates, confirms underdevelopment of the immune system in pigs aged 5 weeks.
2. ABC method proved successful for the existence of lymph cell populations. With optical microscope stained cells differ significantly from those of unstained.
3. Flow cytometry gave us NLM notes, spleen and the peyer plates of the the ilium: CD 45 RA is involved in the largest proportion of animals in the experiment group than the control group of animals. Also marker CD 45 RC is present in greater proportion in the NLM and spleen in the experiment group, while in the ilium PP group in the experiment is expressed as a lower percentage compared with the control group.

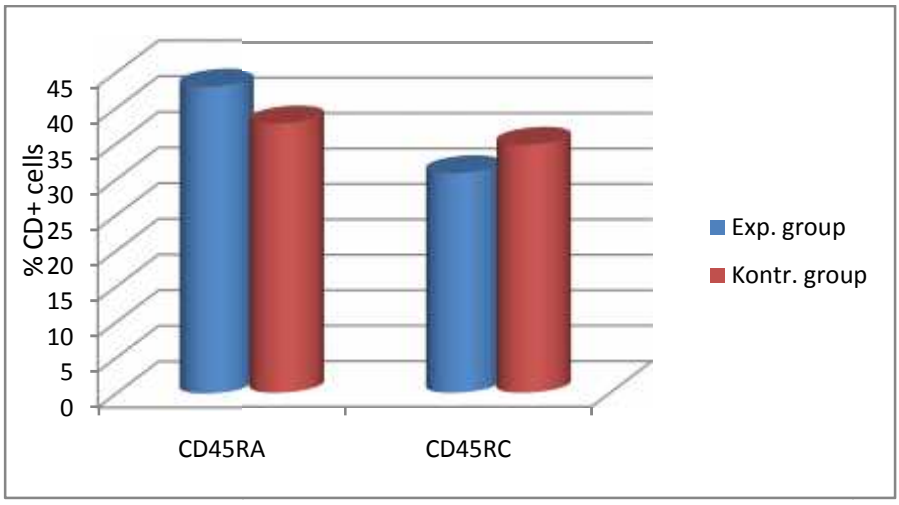

Fig.1.
Percentage of lymphoid cells NQ Peyer plates the ilium of the experimental and control animals aged four week.

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