ASPECTS REGARDING THE ACTIVITY OF THE PHAGOCYTIC MECHANISMS IN IMMUNITY REGULATION OF THE NEW-BORN CALVES

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Abstract

The scientific investigations revealed in this research present the objectives of studying some immunological aspects of the phagocytic activity in immunity regulation in the neonatal period of the new-born calves at the age of 5, 10, 20 and 30 days. As a result of the immunological investigations were revealed various indexes which characterize the activity and intensity of the phagocytic activity, which varies at different age periods. The dynamics of these indexes demonstrates that in the neonatal period, at the calves aged of 5 days, the phagocytic activity constituted 52.33 ± 0.60, compared to the age of 10 days, which constituted 41.67 ± 0.65, which shows a decrease expressed by various aspects of external factors, which acts on the newborn animal in the first days of life. The values of the phagocytic activity at the age of 20 and 30 days constituted 38,56 ± 0,56 and 35,44 ± 0,47, which confirms the decrease of the phagocytic processes at the animals. From the obtained results, it is noticeable that the phagocytic intensity at the neonatal animals at the age of 5 and 10 days determined significant values which constituted 2,32 ± 0,02 and 1,83 ± 0,01, compared to the animals at the age of 20 and 30 days, where these values constituted 1,78 ± 0,01 and 1,57 ± 0,01. The results of the research demonstrated that at the neonatal animals the defense mechanisms are not sufficiently triggered in order to protect against the microorganisms, viruses and other pathogenic agents aggression.

Key words: *Phagocytosis, Phagocytic activity, Phagocytic intensity, Macrophages.*

Introduction

According to the classic conception, is considered that the activity of the immune system has exclusively a beneficial, protective effect on the organism. From this point of view, the immune system is tolerant to its own substances, because it has learnt to recognize them during embryonic life, but it has the property to recognize and differentiate promptly the foreign substances, towards which it activates and removes them from the organism.

The cellular base of the cellular immune response is represented by the phagocytic mechanisms, immunocompetent cells, etc. In this context, the cellular immune response protects the organisms against the aggression of fungi, parasites, viruses and bacteria with intracellular localization. Therefore, they are responsible for the cellular immunity, they express receptors that recognize only short peptide sequences from the protein antigens [1], [3], [4].

The elements of the immune system - macrophages, lymphocytes - T - B with populations, determine the immunological profile or the immune status and results from the complex analysis process of the elements that take part from the immune system. These immunocompetent cells are developing till the animal is born, after which they begin intensively to function, favoring the development of the immune response from the first days of life of the newborn animal.

In its concept, the immune system is one of the most complex from the organism. Its complexity derives from the complicated network structure of communication, cellular and molecular, maintaining permanently the supervision of organisms. They recognize almost limitless variety of foreign cells and molecules, distinguishing them from those of the organism itself. Therefore, they "remember" each infection and at a second exposure to the same pathogen agent, they react more efficiently.

The immune system is so cleverly designed that, if it would be in a perfect functioning condition, it may oppose to any type of aggression. It is consisted from cellular and humoral

components, capable to specifically recognize the antigenic determinants and to bind by them through efficient structures. The antigen (the non-self structure) penetrated in the organism, induces the clonal selection of lymphocytes through a mechanism of the lymphocytes selection, which possess specific receptors, for the non-self structure, after that appearing the cell division, through the"clonal expansion", followed by the increasing number of lymphocytes [2], [5], [6].

The mechanisms through which it operate are external and internal mechanisms. The external mechanisms are represented by skin, mucous membranes and body fluids. These constitute natural barriers that prevent the penetration of pathogens in tissues. When these mechanisms are defeated, it is trying the removal of the pathogen antigens through the internal mechanisms of the nonspecific immunity: additional physiological factors, phagocytosis and inflammation, constituting the immune defense mechanisms, which acts on the defense mechanisms of the host organism.

The study of the immune system components and their intervention in ensuring the organisms homeostasis exposed to harmful external and/or internal environmental factors influence is currently a significant desiderate.

Structural integrity of immunocompetent organs, as of the immunologic postagresional ways of response constitute a 'sine qua non' survival condition of individuals and species [7], [8], [9].

Therefore, the main objectives of these researches constitute the study of the phagocytic mechanisms in regulation of the immunity at the newborn calves.

Material and method

The scientific investigations were performed at the Laboratory of Microbiology from the Faculty of Veterinary Science of the State Agrarian University of Moldova. For realization of the investigations were used samples of blood from the new-born calves aged up to 30 days, in order to determine the immunological blood indixes. The blood samples were collected from the jugular vein with heparin, based on the 0,3 ml of heparin and 10,0 ml of blood, for the purpose of anticoagulation.

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Samples of blood were used for the opsono-phagocytic test constituted from the cellular mechanism of the phagocytosis and the involved cells in this process.

For performing the opsono-phagocytic test was used 1,0 ml of blood stabilized with heparin and 0.1 ml suspension of E. coli from 1.0 ml physiological solution with 500 mln. microbial cells. The tubes were stirred, then incubated in the thermostat during 30 minutes at T-37 ° C and centrifuged at 1500 rot./ min. The supernatant was removed using the Pasteur pipette. Were performed smears colored according to the Romanowsky – Giemsa method, fixed with methanol and colored during 30 minutes. The samples of blood were viewed under a microscope immersion 90.

The test determined the number of microorganisms phagocytosed per 100 neutrophils. The index of activity and intensity of the phagocytosis of the cells was determined as the percentage of neutrophils cells that participate in the process of phagocytosis.

At the same time the phagocytic intensity was determined by the number of the microorganisms incorporated by one neutrophil. The calculation was represented by the report between the sum of the phagocytosed microorganisms devided to the number of the neutrophils which participate at the reaction.

Results and discussion

As a result of the immunological investigation, regarding the study of the immune phagocytic mechanisms in the neonatal period at the new-born calves revealed different indices characteristic to the phagocytic activity and intensity which varies in different age periods.

The indices of the phagocytic activity at the neonatal calves determined significant values at different age periods (Fig. 1). These data reveal that the animals possess resistance to infectious germs. Therefore an important factor of the protecting organism cellular system is represented by the opsono – phagocytic reaction of the leukocytes.

The dynamics of these indexes demonstrates the fact that in the neonatal period at the calves aged 5 days, the phagocytic activity was 52.33 ± 0.60, compared to age 10 days, which constituted 41.67 ± 0.65, which reveals a decrease expressed by various aspects of external factors acting on the newborn animal in the first days of life. Analyzing the dynamic of the indices of phagocytic activity at the age of 20 and 30 days it was found that the values constituted 38.56 ± 0.56 and 35.44 ± 0.47, fact which confirms the decrease of the phagocytic processes at these animals.

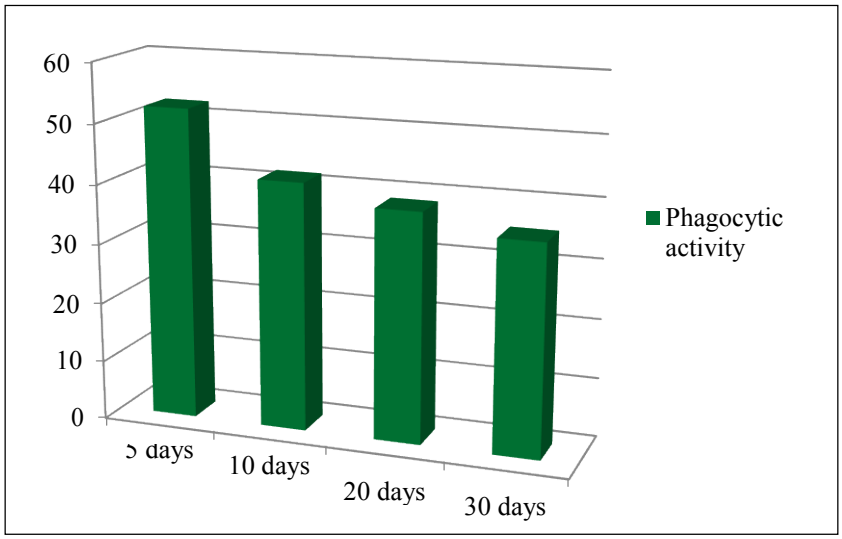

Fig.1. The indices of the phagocytic activity at the new-born calves depending on age

The dynamics of these indexes demonstrates the fact that in the neonatal period at the new-born calves aged 5 days the phagocytic activity constituted,33 ± 0,60, compared to the age of 10 days, which constituted 41,67 ± 0,65, which reveals a decrease expressed by various aspects of the external factors, acting on the newborn animal in the first days of life. Analyzing the dynamics of the phagocytic activity indexes at the age of 20 and 30 days was stated, that the values were 38,56 ± 0,56 şi 35,44 ± 0,47, which confirms the decrease of the phagocytic processes at these animals.

In the immunological aspect, one can see that the phagocytic activity in this period of life of neonatal calves is attributed to the fore neutrophils, the rest being made by the macrophages. Therefore, the phagocytic mechanisms induce phenomena which can be realized in two ways, depending on the bacteria resistance: first way, without opsonizing using the direct interaction between the phagocytic cell and the antigen; and the second way, with the opsonizing which constitute the interaction for which is necessary an additional molecule,

opsonine, which plays an adaptor role between the bacteria and the leukocyte. In this context, the phagocytosis is continuing with the adhesion, after that with the fase when the pseudopodia surround the bacteria. The final faze of distruction offers the final digestion of the bacteria.

The mechanism of the immune response regulation is based on the immune reaction, controled by the regulation systems. In this context in the situation of blocking the regulation mechanisms, the clonal proliferation or the syntheis of the immunoglobulins cannot be limited, which leads to the profound alteration of the immune response, accompanied by installation and evolution of diseases that usually have a fatal end.

Therefore, the cellular activity processess are initiated through the mechanisms and the complexes characterized by the initiation and realization of functions of cells implicated in the immumne response. According to these reserches we can affirm that the immune system cells pass the steps of the cellular cicle.

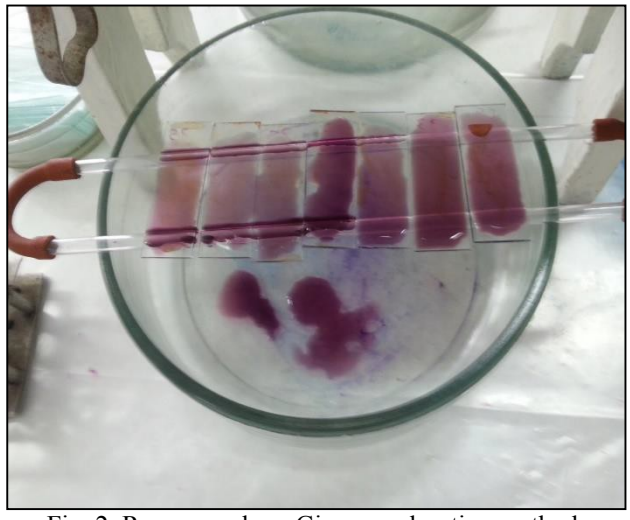

Fig. 2. Romanowsky – Giemsa coloration method.

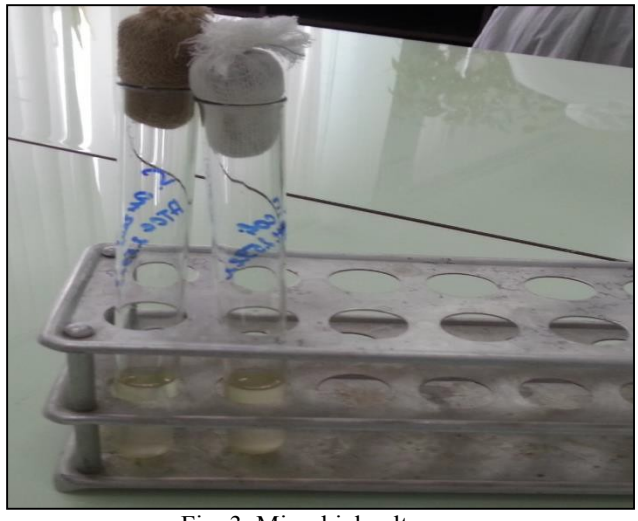

Fig. 3. Microbial cultures.

Important data were registered related to the phagocyticic intensity at the neonatal animals in diverse age periods (Fig. 4)

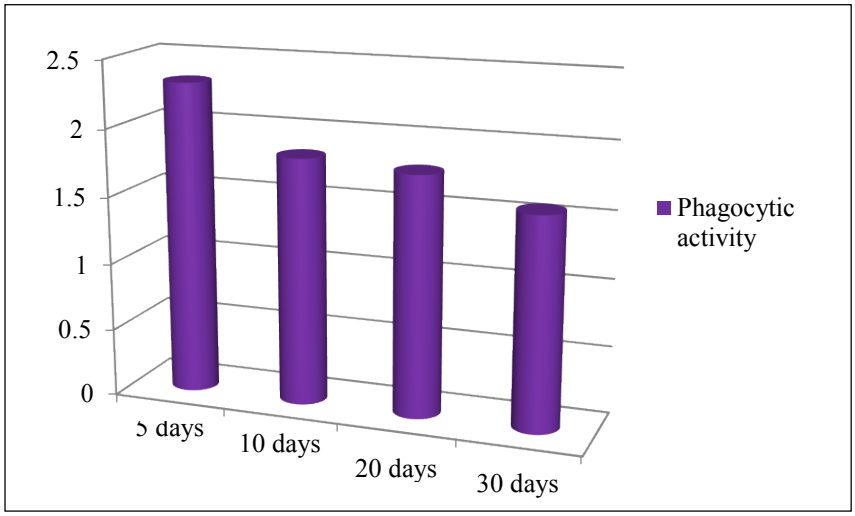

Fig.4. The indices of the phagocytic intensity at the new-born calves depending on age

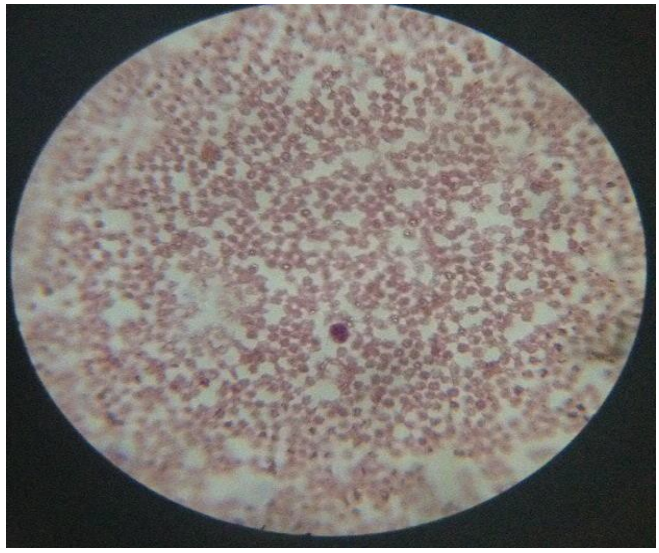

Fig. 5. Opsono-phagocytic test

According to the obtained results, it can be stated that the phagocytic intensity at the neonatal animals with the age of 5 and 10 days determined significant values 2,32 ± 0,02 şi 1,83 ± 0,01, compared to the animals with the age of 20 and 30 days, where these values constituted 1,78 ± 0,01 and 1,57 ± 0,01. Therefore, at the neonatal animals the defense mechanisms are not sufficiently triggered for the protectiom against the aggression of the microorganisms, viruses and other pathogens.

At the same time, these data reveal that the cellular activation processes are initiated through mechanisms characterised by the initiation and realization of the cellular functions

implicated in the immune response. According to these researches we can affirm the fact that the immune system cell, pass the steps of the cellular cycle. From these point of view, the activation of the immune system cells are realized through the signals of the antigen and of a costimulatory molecule represented by the cytokine IL-1. In this aspect are realised other activations of the immune processess as a result of the recognition of the antigen by the molecule BCR.

According to the specialty studies the neutrophils, monocytes and macrophages implicated in this process, constitute the first line of defence against the pathogenic organisms. The elimitation of the bacterial infections through the process of phagocytosis implicates the recruitment of the neutrofils at the level of the blood circluation and from the level of bone marrow by chemotaxis to the place of infection. Thus the neutrophil phagocytic activity is potentiated by the complement system and by antibodies.

The development of immunity and tolerance is supposed to some mechanisms of fine regulation because the immune response against the self antigen or the toleration of a pathogen potential can have unfavorable consequences for life. The regulation of the humoral or cellular immune response is a complex process of modulation where intervene a number of ways through which is mentained the specific defence of the organism at a certain level an with a certain duration, in order to realize the homeostasis and to mentain the health.

Conclusions

1. The nonspecific immunity is considered the first means of defense in the immune response. The mechanisms through which it operates are external and internal, which constitute the main natural barriers, preventing the penetration of the pathogenic antigens in the tissues.
2. The results, regarding the phagocytic activity in this period of life at the neonatal calves is attributed first of all to neutrofils, the rest being performed by macrophages.
3. The phagocytic mechanisms induce phenomena that can be achieved in two ways, they depend on the resistance of bacteria which is assumed to the direct interaction between the phagocytic cell and the antigen and the adapter process between the bacteria and the leukocyte.
4. Phagocytic intensity values at neonatal animals in different age periods determined significant values, fact which confirms that the defense mechanisms are not sufficient to protect against the aggression of microorganisms, viruses and other pathogens.

References

1. Andrieş L., Barba D., Cerneţchi O., Stratan V., 2014: Imunologie clinică. Chişinău: Editura „Tipografia Centrală". 556 p: ISBN 978-9975-53-383-6.
2. Andrieş L., Olinescu A., 1992: Compendiu de imunologie fundamentală. Chişinău: Editura „Ştiinţa". 476 p.
3. Brokaw A., 2013: Immunology: Use Howard Hughes Medical Institute Resurces to Teach. Ohio. 37 p. Disponibil: http: // www.hhmi.org/ biointeractive/ teacher-guide-immunologzy.
4. Cristea V., Crişan M.,2011: Curs de imunologie pentru studenţii facultăţii de medicină. Cluj-Napoca.255 p.
5. Gâjâilă G., 2002 : Imunologie analitică. Aspecte fundamentale şi metodologice. Bucureşti: Editura „ Printech". 224p: ISBN 973-652-583-X.
6. Gâjâilă G., 2003 : Sistemul imunitar la suine. Bucureşti: Editura „Cartea Universitară". 131 p. ISBN 973-86231-7-0.
7. Rosen R Hugo., 2008: Transplantation Immunology: Whatthe Clinician Needs to Know for Immunotherapy Gastroenterology. 134 : 1789-180.
8. Siloşi I., 2014 : Imunologie. Craiova: Editura „SITECH". 266P. ISBN 978-606-11-3717-6.
9. Taşbac A., 2014: Îndrumar pentru laboratorul de imunologie veterinară. Bucureşti: Editura „Larisa", Câmpulung Muscel, 170p. ISBN 978-606-715-271-5.