

THE IMPORTANCE OF PHAGOCYTOSIS IN ASSESSING CELLULAR IMMUNITY AT LAMBS

Rita GOLBAN¹

e-mail: golbanrita@gmail.com

Abstract

The scientific research reflected in these investigations constituted the study of some immunological aspects of the immune defence mechanisms in various periods at lambs. As a result of the immunological investigation, various indices characteristic of phagocytic activity and intensity were revealed. It was found that the phagocytic activity in these periods of the lambs' life is primarily attributed to neutrophils, the rest being carried out by macrophages. At the same time, the investigations have demonstrated that the defence mechanisms of the investigated animals are not triggered enough to protect the aggression of microorganisms, viruses and other pathogenic agents. The results of the study determined that the regulation of the humoral or cellular immune response is a complex modulation process involving a series of means through which the body's specific defence is maintained at a certain level and with a certain duration, in order to achieve homeostasis and preserve health status.

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

The immune response is considered a cellular and humoral defense mechanism, through which the body recognizes what is foreign to itself. In maintaining the phenotypic homeostasis, the animal organism is endowed with various defense mechanisms and is constantly requested by factors of the external environment, which, coming into contact with its defense mechanisms, favor mechanisms against what is non-proper for the cellular constituents, having as resulting in their neutralization and elimination [5,7,9].

The activity of the mechanisms favoring some infections has an important role in the regulation of the immune response. Immunological and autoimmune pathology is of current interest and interests all fields of both human and veterinary medicine. Therefore, the recognition of the self from the non-self is specific to each organism and is the basis of the innate immunological tolerance towards the own immunocompetent cells, favoring immune defense reactions against substances with foreign intervention [1,3,4,6].

Non-specific immunity presents an immediate means of defense in the immune response, and the most important mechanisms are considered to be external and internal. The external mechanisms are represented by the skin, mucous membranes and body fluids. These constitute natural barriers that prevent the penetration of pathogenic agents into the tissues. Once these

mechanisms are defeated, an attempt is made to remove the pathogens through the internal mechanisms of non-specific immunity: phagocytosis, inflammation, physiological factors, constituting the immune defense mechanisms that act complexly on the host organism's defense mechanisms [2,8].

Scientific research on the mechanisms of phagocytosis and its intervention in protecting the human and animal body subjected to the harmful influence of external and internal environmental factors is currently an important topic [10].

For these reasons, the main objectives of these researches are to study the aspects of phagocytosis in the assessment of cellular immunity at lambs.

MATERIAL AND METHOD

The scientific investigations were carried out in the Faculty of Veterinary Medicine in the microbiology and immunology laboratory of the Technical University of Moldova. To carry out the investigations, blood samples from lambs in various age periods were used. Blood samples were collected from the jugular vein with heparin based on the calculation of 0.3 ml of heparin per 10.0 ml of blood for the purpose of anticoagulation. The blood samples were used to perform the opsono-phagocytic test constituted by the cellular mechanism of phagocytosis and the cells involved in this process

¹ Technical University of Moldova

According to specialist studies, the neutrophil cells, monocytes and macrophages involved in this process constitute the first line of defense against pathogenic organisms. The elimination of bacterial infections through the process of phagocytosis involves the recruitment of neutrophils from the bloodstream and from the hematogenous marrow through chemotaxis to the site of infection. Thus, the phagocytic activity of neutrophils is potentiated by the complement system and by antibodies.

To perform the opsono-phagocytic test, 1.0 ml of blood stabilized with heparin and 0.1 ml of E.coli microbial culture suspension from the calculation of 1.0 ml of physiological solution at 500 million microbial cells were used. The tubes were shaken, then incubated in a thermostat for 30 minutes at T-37 C and centrifuged at 1500 rpm. The supernatant was removed by means of a Pasteur pipette. Stained smears were performed according to the Romanovschii-Giemsa method, fixed with methyl alcohol and stained for 30 minutes. Preparations from blood samples were visualized under a microscope, immersion 90. The test determined the number of phagocytosed microorganisms per 100 neutrophils. The index of phagocytic activity and intensity was determined by determining the percentage of neutrophil cells, which participate in the phagocytosis process. At the same time, the phagocytic intensity was determined by the number of microorganisms engulfed by a single neutrophil. The calculation was made by expressing the ratio of the amount of phagocytosed microorganisms to the number of neutrophils participating in the reaction.

RESULTS AND DISCUSSIONS

Immunological investigations, regarding the study of immune phagocytic indices in different age periods at lambs, revealed characteristic indices of phagocytic activity and intensity.

The indices of phagocytic activity at lambs determined significant values in investigated periods of age and time shown in figure 1. These data denote that the animals possess resistance to infectious germs. The important factor of the cellular system of protecting the body is represented by the opsono-phagocytic reaction of leukocytes.

The dynamics of these indices demonstrate the fact that in the neonatal period at calves aged 10 days, 6/12 hours the phagocytic activity was $50.33 \pm 0.60/47.23 \pm 0.54$, compared to the age of 20 days, 6/ 12 hours, which constituted $40.67 \pm 0.65/37.13 \pm 0.31$, which denotes a reduction expressed by

various aspects of the external factors, which act on the newborn animal in the first days of life. Following the dynamics of the phagocytic activity indices at the age of 20 and 30 days, it was found that the values were $40.67 \pm 0.65/37.13 \pm 0.31$, and $38.44 \pm 0.47/34.11 \pm 0.21$, which confirms the reduction of phagocytic processes at these animals.

In immunological aspect, it can be found that the phagocytic activity in this period of life of the lambs is primarily attributed to neutrophils, the rest being carried out by macrophages. Therefore, the phagocytic mechanisms induce phenomena that can be achieved through two ways, depending on the resistance of the bacteria: the first way, without opsonization through the direct interaction between the phagocytic cell and the antigen; and the second way, with opsonization, is the interaction that requires an additional molecule, opsonin, which plays the role of adapter between the bacterium and the leukocyte. In this context, phagocytosis continues with adhesion, then with the phase in which the pseudopods surround the bacterium. The final destruction phase provides complete digestion of the bacterium.

Relevant data were recorded, regarding the phagocytic intensity at the investigated lambs in various periods of age and time reported in figure 2.

From the obtained results, it can be found that the phagocytic intensity in neonatal animals at the age of 10 days, 6/12 hours determined significant values $2.13 \pm 0.01/1.95 \pm 0.01$, compared to animals aged 20 and 30 days, 6/12 hours, where these values were $1.83 \pm 0.02/1.82 \pm 0.01$ and $1.56 \pm 0.01/1.45 \pm 0.01$. Therefore, in neonatal animals, the defense mechanisms are not triggered enough to protect the aggression of microorganisms, viruses and other pathogenic agents.

The development of immunity or tolerance is subject to fine-tuning mechanisms because the immune response to self-antigens or the tolerance of a potential pathogen can have unfavorable consequences for life. The regulation of the humoral or cellular immune response is a complex process of modulation involving a series of means through which the body's specific defense is maintained at a certain level.

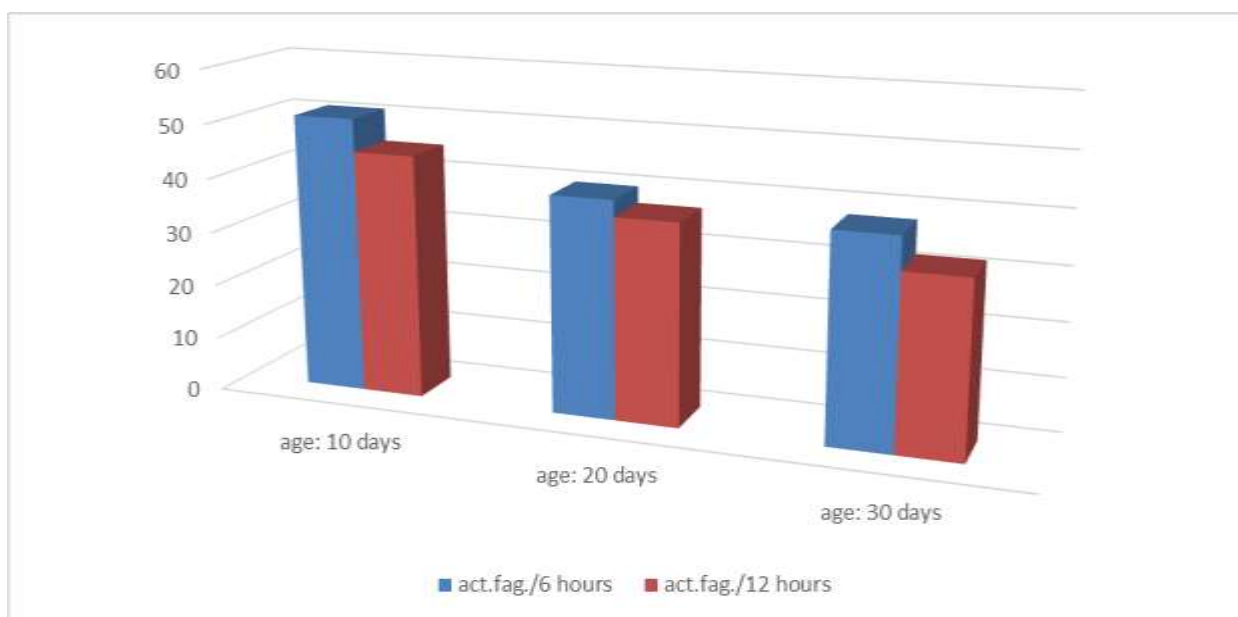


Figure. 1. Indices of phagocytic activity at lambs at different ages and time periods
Source: elaborated by the author

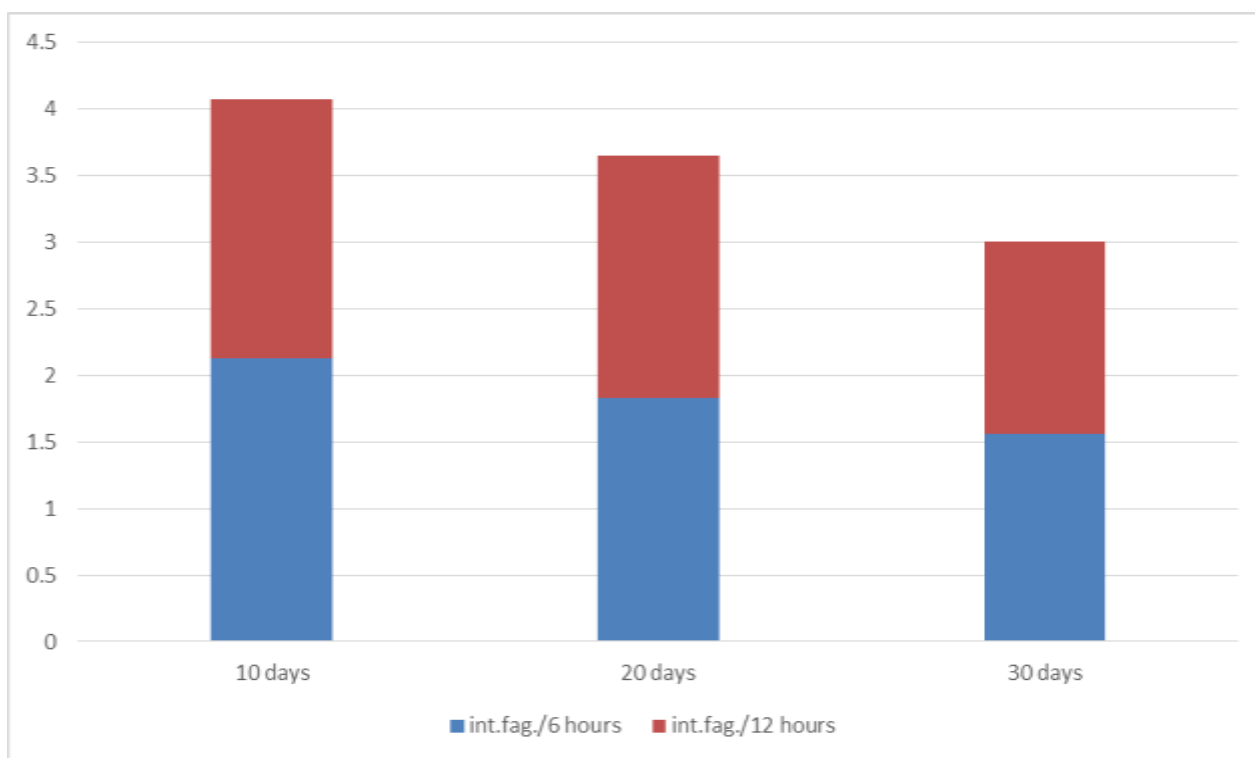


Figure. 2. Indices of phagocytic intensity at lambs at various age periods and time periods
Source: elaborated by the author

CONCLUSIONS

1. The first defense weapon in the immune response is non-specific immunity through external and internal mechanisms, which, preventing the migration of pathogenic germs in the animal's body.

2. The results regarding the phagocytic activity in different age periods of the lambs are characteristic of the activity of the phagocytic cells and their mechanisms.

3. The phagocytic mechanisms depend on the resistance of the bacteria through direct contact between the phagocytic cell/antigen and the bacteria/leukocyte process.

4. The activity of the phagocytic intensity at lambs in different age periods determined significant values, which confirms that the defense mechanisms are not sufficient to protect the aggression favored by various pathogenic agents.

REFERENCES

- [1] **ANDRIEȘ, L., 2014.** Clinical immynology. Chișinău: Central Typography, 556p. ISBN 978-9975-53-383-6.
- [2] **ANDRIEȘ, L., OLINESCU, A. 1992.** Compendium of Fundamental Immunology. Chișinău: Știința, 476 p.
- [3] **BROCAW, A., 2013.** Immunology: Use Howard Hughes Medical Institute Resurces to Teach. Ohio. 37 p. Disponibil: // www.hhmi.org/biointeractive/teacher-guide-immunology.
- [4] **CRISTEA, V., CRIȘAN, M. 2011.** Immunology course for the students of the Faculty of Medicine. Cluj – Napoca, 2011, 255p.
- [5] **GĂJĂILĂ, G. 2003.** The immune system at pigs. Bucharest: Cartea Universitară, 131p. ISBN 973-86231-7-0.
- [6] **GĂJĂILĂ, G. 2002.** Analytical immunology. Fundamental and methodological aspects. Bucharest: Printech, 224p. ISBN 973-652-583-X.
- [7] **ROSEN, R., 2008.** Transplantation Immunology: What the Clinician Needs to Know for Immunotherapy Gastroenterology. 134:1789 – 180.
- [8] **SILOȘI, I. 2014.** Immunology. Craiova: SITECH, 266p. ISBN 978-606-11-3717-6.
- [9] **SILOȘI, I. 2013.** Laboratory investigations in clinical immunology. Craiova: Ed. a II-a, 241p.
- [10] **TĂȘBAC, A. 2014.** Guide for the Veterinary Immunology Laboratory. Bucharest: Larisa, Câmpulung Muscel, 170p. ISBN 978-606-715-271-5.