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THE ROLE OF NEUTROPHIL ON SUBCLINICAL MASTITIS IN COWS

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Abstract

It has been reported that the neutrophils are actively involved in the local udder immune defense. The aim of this study was to investigate the role of milk neutrophils in subclinical mastitis in cows. The research carried out on 70 cows at the farms of the Khmelnitsky Region Ukraine. Cows were divided into experimental (n = 37) and control (n = 33) groups. The indicators of the cytochemical reactivity of oxygen-independent and oxygen-dependent factors of protection on milk phagocytes, cell migration activity index and correlation were evaluated. The results were expressed as mean ± SD and percentage. Changes in the composition of cells were observed: increase of neutrophils ($P < 0.01$), monocytes ($P < 0.01$), and lymphocytes ($P < 0.05$). The indicators like cationic proteins (CP), myeloperoxidase (MPO), reaction neutrophils with nitro-blue tetrazolium (NBT), activation of neutrophils index (ANI) and cytological index (CLI) differed significantly in subclinical mastitis in cows in relation to healthy control group. The neutrophils activation occurred by initiation of respiratory burst, which triggered apoptosis induced by antimicrobial activity. Alterations on the cells migration activity index were observed in subclinical mastitis in cows. Based on results we can suggest that determination of the parameters related to milk neutrophils and the behavior of cell migration could contribute more information to subclinical mastitis diagnosis.

Introduction

Cow's mastitis is the inflammatory pathology of udder dairy cattle, causing significant economic damage (HEIKKILÄ et al. 2018). An important role in this belongs to the body's immune system, which supports homeostasis and responds first to all changes in physiological constants (GARZONI and KELLEY 2009, GÜNTHER et al. 2016). Moreover pathogenesis

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of mastitis depends on numerous etiological factors (MUSHTAQ et al. 2018, RABE et al. 2020). Biological mechanisms of homeostasis. which carry out the control, regulation and adaptation of the organism to changes in environmental conditions (BROZ et al. 2016, YOUSSIF et al. 2019).

The pathogenesis of mastitis and the manifestation of immune responses depend on numerous etiological factors (DERVISHI et al. 2015). The leading etiological factor of mastitis is the microbial factor, which by its virulent and pathogenic properties determines the nature of manifestation and course of the pathological process (GUERRERO et al. 2015). Thus in the body there is a functional restructuring in the local immune protection of the udder (GUO et al. 2015, ZHELAVSKYI 2017). Immunocompetent cells (neutrophils, monocytes, lymphocytes and histiocytes) is involved primarily in the fight against pathogenic microorganisms (JORGENSEN et al. 2016, DERAKHSHANI et al. 2018). The occurrence of mastitis depends on the functional state of the mammary gland, innate resistance and the state of organism (PETZL et al. 2018).

Phagocytes have a variety of defense mechanisms (KRUGER et al. 2015). Particular attention should be paid to the investigation of extracellular factors (oxygen-dependent, oxygen-independent, NETosis) and the investigation of apoptosis cells (LIU et al. 2014, SHI et al. 2015, ZHELAVSKYI 2019).

The study of immunological mechanisms of cow mast development in the future will become the basis of new diagnostic tests and effective methods of treatment. Therefore, the study aim was to investigations the functional of neutrophil of milk in cow's which subclinical mastitis.

Materials and Methods

The research carried out on 70 cows of *Ukrainian black-and-white milk breeding* at the farms of the Khmelnitsky Region Ukraine. Part of the experiments were carried out in a specialized laboratory of mammalian reproductive immunology of the Faculty of Veterinary Medicine of the State Agrarian and Engendering University in Podilya.

Animals' Criteria

All cows were divided into experimental and control (healthy animals, $n = 33$) groups. Experimental group ($n = 37$) formed from cows with subclinical mastitis (SCM). Diagnosis of mastitis made on the basis of cytological diagnosis of milk (Lactoscan SCC, Milkotronic Ltd, Bulgaria). This investigation approved according to the Law of Ukraine "On the Protec-

tion of Animals from Cruel Treatment" (No. 3447-IV of February 21, 2006) and according to the requirements of the European Convention for the Protection of Pet Animals (ETS No. 125, Strasbourg, 13/11/1987). All experiments were carried out with the Ethical Permit at the State Agrarian and Engineering University in Podilya, Ukraine. All animal manipulations were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for experimental and scientific purposes (Strasbourg, 18 March 1986).

Immunology Analysis

In the cytochemical investigation of phagocyte reactivity in milk, the state of Oxygen-dependent phagocytic protection factors. Determined: myeloperoxidase (MPO) activity and reaction neutrophils with nitro-blue tetrazolium (NBT). Oxygen-dependent cellular protection mechanisms were determined by the activity of cationic proteins (CP) (SNOW and LENARDO 2019). In the experiment, it was determined the of activation of neutrophils index, ANI), index cytological (ICL), lymphocyte migration activity index (LMAI), neutrophils migration activity index (NMAI), index of macrophage transformation (IMT) and lymphocyte/granulocyte ratio – LMR (ZHELAVSKYI 2019). Apoptosis of neutrophilic granulocytes was studied for characteristic changes in the nucleus (pyknosis, rexis, vacuolization, fragmentation), cytoplasm (vacuolization and toxic vacuolation), cytolysis (cell disintegration) and size reduction (TAYLOR et al. 2007).

Statistical Analysis

The values in this investigation are presented as mean ± SD. The data in this investigation are given as the mean ± SD. Data were analyzed by one-way analysis of variance (MANOVA). Differences were considered statistically significant at a *P*-value of less than 0.05. Biometric analysis and interpretation of the obtained results were performed using statistical software Stastistica v. 12.6 (StatSoft, Inc., USA, 2015).

Results and Discussion

In Table 1 shows the cytogramme of the secret mammary gland of cows which subclinical mastitis. Inflammation udder manifested by an increase in SCC ($P < 0.01$). Changes in the composition of individual cell populations were also notes: increased the population of neutrophils

($P < 0.01$), monocytes ($P < 0.01$) and lymphocytes ($P < 0.05$). This due to the increase in neutrophil migration activity ($P < 0.01$) and the activation of macrophage transformation processes (Figure 1). Such changes occurred as a result of a microbial attack. Neutrophils of the peripheral blood began to was active in the inflammation zone (Figure 2).

Table 1

Changes in the cytologic composition of the mammary gland of cow's at subclinical mastitis (mean ± SD)

Value	Milk somatic cells						
	Somatic cell counts ($\cdot 10^3$)	leucocytes [%]				histiocytes [%]	epithelial cells [%]
		neutrophils	lympho-cytes	monocytes	total		
Healthy animals (control, n = 33)	255.72±17.5	44.69±0.58	3.87±0.33	1.93±0.24	50.51±1.05	4.18±0.10	45.50±0.89
Subclinical mastitis (n = 37)	3819.23±76.36**	50.73±1.34**	2.33±0.43*	4.28±0.51**	57.26±1.22**	8.2±0.21**	34.51±1.38**

Explanation: n – number; * – $P < 0.05$; ** – $P < 0.01$

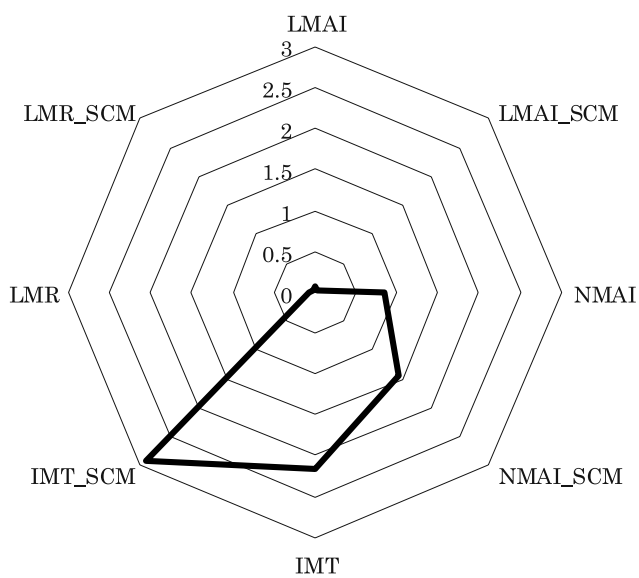

Fig. 1. Changes in cytological parameters in subclinical mastitis (SCM) of cows: LMAI – lymphocyte migration activity index; NMAI – neutrophils migration activity index; IMT – index of macrophage transformation; LMR – lymphocyte/granulocyte ratio

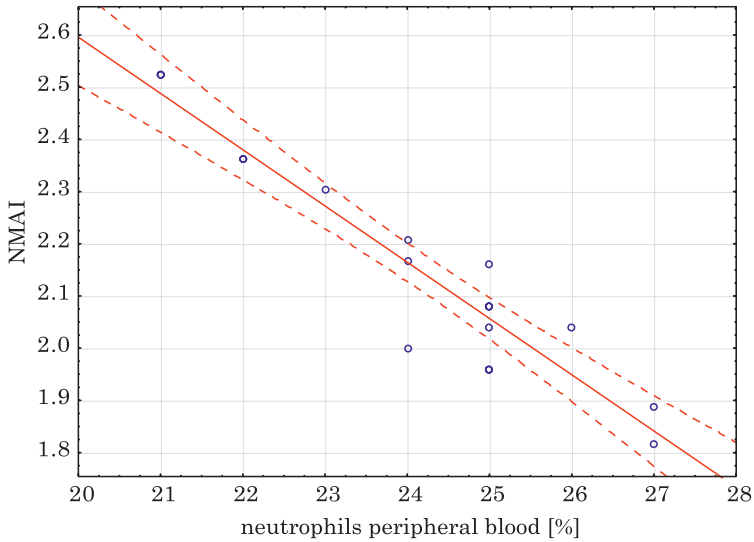

Fig. 2. The plot shows a correlation (r = 0.94) between NMAI – neutrophils migration activity index of cows which subclinical mastitis and the number neutrophils of peripheral blood

Antimicrobial reactivity oxygen-independent factors of protection of local immunity of a mammary gland are shows in Table 2. In the milk of cows at subclinical mastitis increases the activity of the cationic protein of neutrophils ($P < 0.001$).

Table 2

Changes in cytochemical reactivity oxygen-independent factors of protection of cow's breast phagocytes at subclinical mastitis (mean ± SD)

Value	Cytochemical reactivity CP		
	CP [%]	ANI	CLI (%)
Healthy animals (control, n = 33)	57.06±0.65	1.28±0.06	2.88±0.08
Subclinical mastitis (n = 37)	64.63±0.48***	1.04±0.04*	3.01±0.04**

Explanation: n – number; CP – cationic proteins; ANI – activation of neutrophils index; CLI – cytological index; * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$ vs healthy animals

The pathogenesis of inflammation also noted the activation of oxygen-dependent mechanisms of phagocytes (Table 3). Antimicrobial mechanisms of phagocyte protection (MPO, $P < 0.05$, NBT, $P < 0.001$) were also activated in the milk of experimental animals. In the pathogenesis of SCM was clearly dominated by induction of respiratory burst (NBT – test).

Table 3

Indicators of cytochemical reactivity of oxygen-dependent factors of protection of cow's mammary gland phagocytes in subclinical mastitis (mean ± SD)

Value	MPO			NBT − test		
	MPO [%]	ANI	CLI [%]	NBT [%]	ANI	CLI [%]
Healthy animals (control, n = 33)	66.81±0.68	2.80±0.17	2.16±0.07	19.12±0.89	0.44±0.02	2.24±0.08
Subclinical mastitis (n = 37)	77.36±0.94**	1.52±0.72***	3.7±0.05***	74.18±0.69***	1.46±0.05***	3.4±0.06**

Explanation: n – number; MPO – myeloperoxidase; NBT − reaction neutrophils with nitro--blue tetrazolium; ANI − activation of neutrophils index; CLI − cytological index; * − $P < 0.05$; ** − $P < 0.01$; *** − $P < 0.001$ vs healthy animals

Triggering the antimicrobial activity of neutrophils can also affect cell apoptosis (TAYLOR et al. 2007). We also found a significant increase in the number of neutrophils with signs of apoptosis (apoptosis index 42.09±0.83% vs 35.39±0.57% in control). The number of neutrophils granulocytes cells with the vacuolization of the nucleus, zeiosis and the toxic vacuolization of the cytoplasm increased. Showed signs of cytolysis (plasmolysis) of cells.

The researchers are constantly faced with problems of interpretation of the obtained results (GEORGIEV 2008, DERVISHI et al. 2015). This which is related to the dynamic changes somatic cells, periods of lactation, daily fluctuations in their number, breed and individual characteristics of animals (BORTOLAMI et al. 2015, HEIKKILÄ et al. 2018). Thus, cytochemical studies will certainly give researchers a new search for the diagnosis of subclinical mastitis in cows (HEIKKILÄ et al. 2018).

The role of innate neutrophils in immunity and mastitis development is only the beginning to evaluate the physiological functions of apoptosis (RONGVAUX et al. 2014, MALTEZ and MIAO 2016, ZHELAVSKYI 2019). The investigation of the cascade of immune responses and the role of apoptosis is important in determining the physiological constants of immunity (GUERRERO et al. 2015, YOUSSIF et al. 2019). This fact clearly confirmed that inflammatory reaction occurring and against the background of activation of neutrophil migration (ZHELAVSKYI 2017). Obviously, neutrophilic granulocytes that migrated into the udder parenchyma isolated a number of cytokines, enzymes.

The presented investigation shows the process of attraction and phagocytosis of apoptotic neutrophil granulocytes by activated macrophages of mammary gland (LIU et al. 2016, PETZL et al. 2018). Changes occur

together with metamorphoses of the nucleus (pycnosis, rexis, vacuolization), cytoplasm (wrinkles), when the cells lose their specific granularity. Zeiosis of the membrane is one of the metamorphic signs of cell apoptosis. It is manifested by the projection (deformation) of the cell wall. Such projections of the membrane further form apoptotic bodies (vesicles), which are the remains of organoids surrounded by the membrane. Macrophages recognize and phagocytize apoptotic cells through special receptors that interact with external phosphadylserine (RONGVAUX et al. 2014, KRUGER et al. 2015). Thus, it can be concluded that under subclinical mastitis is active migration from the bloodstream to the area of pathological process of neutrophils, which actively destroy pathogenic microorganisms, while being exposed to inflammatory mediators, cytokines, microbial toxins and a number of other substances that reduce their cytochemical reactivity (BROZ et al. 2016).

Migration from the bloodstream of neutrophils leads to and activation of antimicrobial defense mechanisms has led to the release of inflammatory mediators. This including also active excretion into the extracellular space of the active forms of Oxygen (GUO et al. 2015). This made it possible to launch a program of self-destruction of neutrophils (apoptosis) in the parenchyma of the mammary gland (MUSHTAQ et al. 2018, ZHELAVSKYI 2019). Probably microphages also transmit certain signals to the cells of the peripheral bloodstream, so a certain proportion of neutrophils already undergo apoptosis without reaching the pathological process.

Conclusion

The results obtained study indicate that subclinical mastitis of cow's accompanied by intense antimicrobial reactivity of neutrophils. The neutrophils activation occurred by initiation of respiratory burst, which trigger apoptosis induced by antimicrobial activity. Based on results we can suggest that determination of the parameters related to milk neutrophils and the behavior of cell migration could be contribute with more information to subclinical mastitis diagnosis. Correction of the immune responses of local mammary gland immunity may be useful in the development of adequate methods of treatment of cows with mastitis.

Conflict of interest. The author declare that there is no conflict of interest.

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