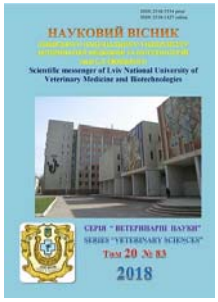

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Changes in the immunobiological reactivity of the organism of cows in the pathogenesis of mastitis

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Immunobiological aspects of the pathogenesis of mastitis of cows of the Ukrainian black-and-white breed are considered in the article. Based on the relevance of the topic, the aim of our work was to study the functional state of nonspecific immunobiological resistance and the specific immunobiological reactivity of the cows' organism during the development of mastitis. The features of the manifestation of immune reactions in the organism of animals in the development of subclinical and purulent-catarrhal mastitis have been studied. Clinical and experimental studies were conducted in Ukrainian farms (Khmelnytsky and Vinnytsia region). Laboratory studies were carried out in the specialized laboratory of immunology of animal reproduction of the Faculty of Veterinary Medicine of the Podilsky State Agrarian and Technical University (Ukraine). Three groups of animals were formed to conduct clinical and experimental studies. As a result, it was found that subclinical mastitis of cows is accompanied by a change in the immunobiological reactivity. Purulent-catarrhal mastitis in cows was manifested by significant changes in the parameters of nonspecific immunological reactivity. The pathological process was accompanied by a sharp decrease in bactericidal activity of blood serum ($P < 0.01$), as well as by suppression of phagocytic reactivity of immunocompetent blood cells. In parallel with this, there was an increase in lysozyme activity of blood serum ($P < 0.01$), which was associated with active degranulation and neutrophil lysis. Obviously, microphages actively migrate to the zone of the pathological process and exhibit active phagocytosis, which was accompanied by partial excretion of cytoplasmic lysozyme. In the peripheral blood of cows with subclinical mastitis, the number of reactive microphages increased sharply ($P < 0.001$). Simultaneously, the number of activated phagocytes with myeloperoxidase granules also increased in the peripheral blood ($P < 0.01$). Activation of intra-leukocyte lysozyme phagocytic cells was less intensive. Subclinical udder pathology was accompanied by an increase in the number of degranulated cells ($P < 0.001$), which is one of the specific properties of cytomorphological changes in programmed death (apoptosis). Subclinical inflammation of the mammary glands mastitis of cows was accompanied by a certain decrease in the number of T-lymphocytes ($P < 0.001$). Clinical and experimental studies have shown that subclinical and purulent-catarrhal mastitis of cows undergo significant changes in systemic immunity. In the pathophysiological model of subclinical and purulent-catarrhal mastitis, the functional state of the T-link of specific immunity was disturbed, the bactericidal activity of blood serum and phagocytosis were suppressed, which occurred against the background of changes in the cytochemical reactivity of phagocytic cells circulating immune complexes and molecules with an average molecular weigh.

Key words: *cows, mammary gland, lactation, mastitis, antimicrobial potential of phagocytes, lysozyme of intraleucocytes, lysosomal cationic protein, myeloperoxidase, NBT-test, immune homeostasis.*

Introduction

Mastitis of cows is a common disease of dairy cattle breeding, which causes serious economic damage to the industrial economies of the CIS countries and Europe (Hamilton et al., 2006; Kurjogi and Kaliwal, 2014; Abebe et al., 2016). Nowadays, modern methods of diagnosis, prevention and therapy of mastitis have been developed and introduced, but in spite of this, the immunological aspects of the pathogenesis of breast pathology have not been studied yet. It is well-known that the pathogenesis of mastitis involves complex mechanisms of development (Green et al., 2007; Singh et al., 2011; Al-Farha et al., 2017), but immune reactions play the main role at it (Yablonskyi and Zhelavskyi, 2007; Thompson-Crispi et al., 2014; Wang et al., 2014). The cascade of immunological processes determines the peculiarities of the manifestation of the disease, the prediction and outcome of the

pathology (Wu et al., 2015; Kempf et al., 2016; Sato et al., 2017).

The aim of our work was to study the functional state of nonspecific immunobiological resistance and the specific immunobiological reactivity of the cows' organism during the development of mastitis.

Material and methods

Clinical and experimental studies were conducted in Ukrainian farms (Khmelnytsky and Vinnytsia region). Laboratory studies were carried out in the specialized laboratory of immunology of animal reproduction of the Faculty of Veterinary Medicine of the Podilsky State Agrarian and Technical University (Kamyanets-Podilsky, Khmelnytsky Region, Ukraine). The experiments were conducted on cows-analogues of the Ukrainian black-and-white dairy breed using the method of groups and periods.

Three groups of animals were formed to conduct clinical and experimental studies. In the first, control group (n = 32) there were clinically healthy cows. The second experimental group (n = 58) consisted of animals with subclinical mastitis. The third group (n = 28) consisted of cows with a clinical diagnosis – purulent-catarrhal mastitis.

A complex study of the immunobiological status was carried out during the testing of non-specific immunobiological resistance (phagocytosis; cytochemical reactivity (NBT-test, MPO, LCP, ILL), bactericidal activity of serum (BASB), lysozyme activity of blood serum (LASB)) and parameters of specific immunobiological reactivity (T-($CD3^+$) and B-($CD22^+$)) lymphocytes, circulating immune complexes (CIC): large, medium CICm (11-19 S) and low molecular weight; molecules of average molecular weight, SM); other immunobiological parameters (LGI, LII, RNN) (Yablonskyi and Zhelavskyi, 2007).

Biometric analysis of the obtained research results and interpretation of data were carried out using statistical program Statistica v. 10.

Results and discussion

Cellular and humoral immune defense factors that underlie the body's immune homeostasis reflect the state of regulatory and effector mechanisms of immune defense (Yablonskyi and Zhelavskyi, 2008; Yablonskyi and Zhelavskyi, 2009; Blum et al., 2015; Kempf et al., 2016; Lombardini et al., 2017; Ndhlovu and Masika, 2017; Cao et al., 2018). The studies carried out in this direction have shown that the parameters of immune homeostasis change in the pathogenesis of mastitis.

Clinical and experimental studies have established that subclinical mastitis of cows is accompanied by a change in the immunobiological reactivity. Initially, the changes were reflected in the violation of the lymphocyte-granulocyte ratio (LGI, 0.73 ± 0.07, P < 0.01), which was more aggravated by the development of a purulent-catarrhal inflammatory process (0.61 ± 0.03, P < 0.01). Along with this, the leukocyte intoxication index (LII, figure 1) was changed – the marker of the depth of endogenous intoxication by metabolites of inflammation (microbial toxins, cellular elements, peptides, etc.).

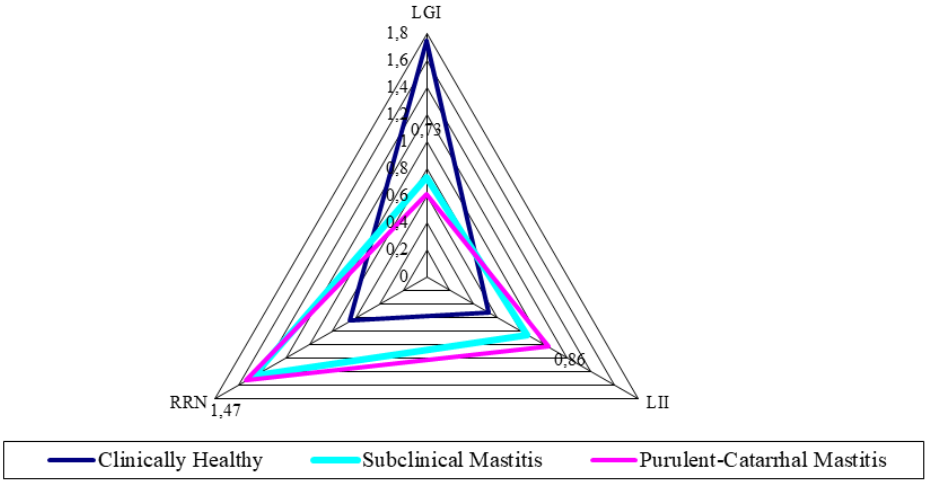

Fig. 1. Changes in hematological indices in subclinical and purulent-catarrhal mastitis of cows (M ± m)

Subclinical inflammatory process in the cows bodies showed a sharp decrease in the level of bactericidal activity of blood serum (48.31 ± 1.28 vs. 53.75 ± 2.37%, P < 0.01) and a slight increase in lysozyme activity of blood serum (24.34 ± 1.55 to 27.15 ± 1.10%). Inflammatory reaction of the organism was also manifested when the phagocytic index decreased to 5.35 ± 0.47; phagocytic number to 4.35 ± 0.45 and total phagocytic capacity from 29.70 ± 2.11 to 23.35 ± 3.80, which also indicates an initial dysfunction in the phagocytic protection system of immunity.

Purulent-catarrhal mastitis in cows was presented by significant changes in the parameters of nonspecific immunological reactivity. The pathological process was accompanied by a sharp decrease in bactericidal activity of blood serum (P < 0.01), as well as by suppression of phagocytic reactivity of immunocompetent blood cells. In parallel with this, there was an increase in LASB (P < 0.01). The phenomenon of increased serum lysozyme activity was associated with active degranulation and neutrophil lysis. Obviously, microphages actively migrate to the zone of the pathological process (parenchyma of the breast) and exhibit active phagocytosis,

which was accompanied by a partial excretion of cytoplasmic lysozyme.

Serial immunological studies determined that subclinical mastitis is accompanied by activation of antimicrobial reactivity of neutrophils in the NBT-test.

In the peripheral blood of cows with subclinical mastitis, the number of reactive microphages increased sharply (by 2.6 times, up to 17.58 ± 0.64%, P < 0.001). This metabolic reaction of antimicrobial enzyme systems was carried out against the background of level activation of the cytological index. In parallel with this, the number of activated phagocytes with myeloperoxidase granules also increased in the peripheral blood (from 66.12 ± 0.94 to 74.58 ± 1.15, P < 0.01). The value of the SPI was also significantly higher (P < 0.001) than the control.

The activation of intra-leukocyte lysozyme phagocytic cells was less intensive. Subclinical udder pathology was accompanied by an increase in the number of degranulated cells (up to 0.57 ± 0.01, P < 0.001), which is one of the specific properties of cytomorphological changes in programmed death (apoptosis). The total index of cytochemical potential in subclinical inflammation of the udder cows was 0.73 ± 0.07, which is a sign of the prevalence of Oxygen-dependent defense factors in the genesis of this pathology development (Zhelavskyi, 2004; Zhelavskyi, 2005).

It was found in the biometric processing of data sets that there is a direct correlation (r = 0.82) between LASB and the neutrophil degranulation index (IDN, Figure 2) in subclinical mastitis, which convincingly proves that in the pathogenesis of subclinical mastitis, neutrophilic granulocytes are actively degranulated, releasing a significant amount of lysozyme to the extracellular space.

Subclinical mastitis of cows was also manifested by changes in specific immunobiological reactivity. Subclinical inflammation of the mammary glands mastitis of cows was accompanied by a certain decrease in the number of T-lymphocytes (from 53.40 ± 0.83 to 47.08 ± 1.01%, P < 0.001).

A purulent-catarrhal inflammatory reaction was manifested by a sharp suppression of $CD3^+$ immunocompetent cells of the cellular defense link (41.07 ± 1.65%, P < 0.001). In the pathogenesis of mastitis, there was also a decrease in the proliferative activity of blasts T- and B-lymphocytes (P < 0.001).

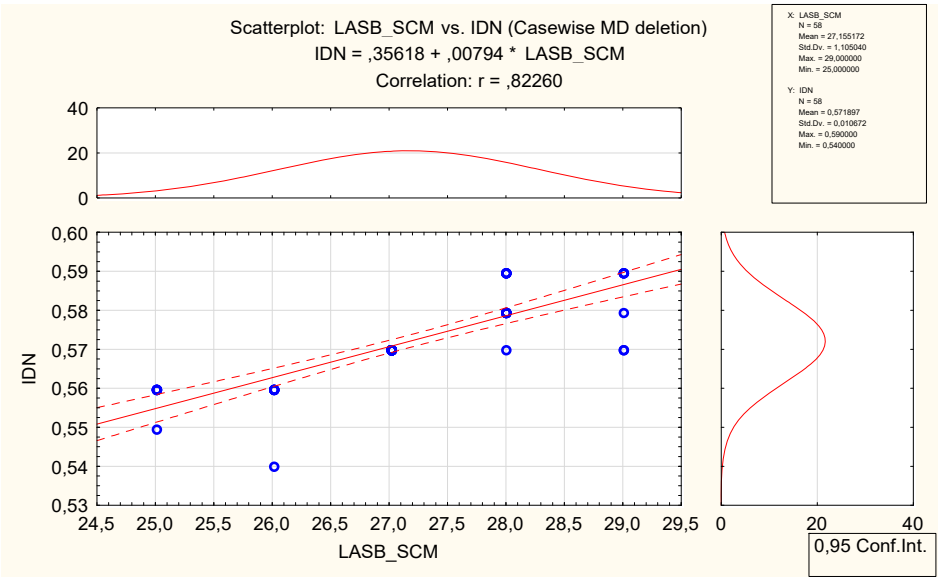

Fig. 2. Correlation (r = 0.82) between lysozyme activity of serum and neutrophil degranulation index

As it is known, autoantigene reactions play an important role in immune reactions – the process of formation of antibodies on the cellular and humoral elements of one's own organism (Yablonskyi and Zhelavskyi, 2007; Yablonskyi and Zhelavskyi, 2008). Usually, autoantigenic reactions in the body are controlled by immunocompetent cells, which forms the basis of immune homeostasis (Zhelavskyi, 2015; Zhelavskyi, 2017). In the literature, domestic and foreign scientists have repeatedly pointed out the pathogenetic effect of circulating immune complexes and medium-molecular molecules on the system of local and systemic immunity in the pathology of the mammary gland of animals (Zhelavskyi, 2011; Zhelavskyi, 2012; Ceniti et al., 2017). Excessive formation and imbalance of CIC and SM often leads to suppression of the functional state of immunocompetent cells and the development of immunocomplex inflammation (Zhelavskyi, 2008; Zhelavskyi, 2009; Ceniti et al., 2017; Pang et al., 2017).

Our studies noted some changes in antigenic reactions in the body of sick cows having the pathology of the breast. In subclinical mastitis, there was a significant (almost 1.5-fold, P < 0.001) increase in the level of circulating immune complexes (CIC) with an average molecular weight (CICm) and a nearly triple increase in the content of medium molecular molecules (up to 3.60 ± 0.25 vs. 1.16 ± 0.07, P < 0.001). These immunological disorders are diagnostic marker indicators of the increase in endogenous intoxication by metabolites of inflammation. A direct correlation was established (r = 0.72, P < 0.001) between the content of medium-molecular immune complexes and medium molecules, which proves the active participation of inflammatory metabolites in autoimmunization of the organism (Figure 3).

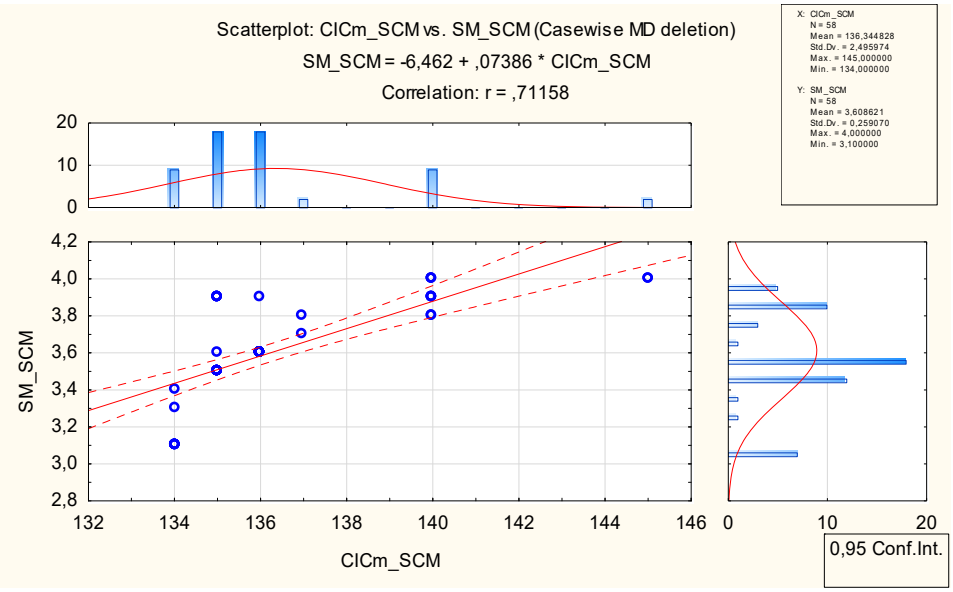

Fig. 3. Correlation (r = 0.72, P < 0.001) between the content of medium-molecular immune complexes and medium molecules

With purulent-catarrhal mastitis, there was a sharp increase in the CIC of medium-molecular ClCm (11–19S) with a low clearance of elimination (up to 220.44 ± 4.56, P < 0.001). It is well known that ClCm has the greatest pathogenicity and often provokes autoantigenic overload in the body of sick animals.

Conclusions

Clinical and experimental studies have shown that subclinical and purulent-catarrhal mastitis of cows undergo significant changes in systemic immunity. In the pathophysiological model of subclinical and purulent-catarrhal mastitis, the functional state of the T-link of specific immunity was disturbed, the bactericidal activity of blood serum and phagocytosis were suppressed, which occurred against the background of changes in the cytochemical reactivity of phagocytic cells (NBT-test, MPO, LCP, ILL) circulating immune complexes and molecules with an average molecular weight.

Subclinical inflammation of the mammary glands mastitis of cows was accompanied by a certain decrease in the number of T-lymphocytes. A purulent-catarrhal inflammatory reaction was manifested by a sharp suppression of $CD3^+$ immunocompetent cells of the cellular defense link. In the pathogenesis of mastitis, there was also a decrease in the proliferative activity of blasts T- and B-lymphocytes.

There was a significant increase in the level of circulating immune complexes in subclinical mastitis with an average molecular weight and a nearly triple increase in the content of medium molecular molecules.

There was a sharp increase with purulent-catarrhal mastitis in the CIC of medium-molecular ClCm (11–19S) with a low clearance of elimination, which has greatest pathogenicity and often provokes autoantigenic overload in the body of sick animals.

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