Immunobiological status of the body of cows during mastitis

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The work reveals the immunobiological aspects of lactation of cows and changes in immunobiological reactivity in the development of mastitis. The authors present modern scientific data on the local immune protection of the mammary gland of cows. Main stages of ontogenetic development of cellular immunity of the mammary gland of cows were traced during clinical and experimental studies. The number of somatic cells in the secret of the mammary gland of the primates was dependent on the period of the functioning of the mammary gland. In the cytology of colostrum mostly (56.00 ± 1.90%) neutrophil granulocytes were predominant, in the middle period of lactation (3–5th month) the proportion of epithelial cells increased (from 29.51 ± 2.17 to 49.59 ± 1.94%), during the launch period, the population of polymorphonuclear neutrophil granulocytes was changing as well, which virtually recovered to the original level and increased during the dry period. However, at the end of lactation, during the onset and dry, with the development of involutionary processes in the mammary gland, a sharp decrease in cytochemical reactivity of intracellular lysozyme of phagocytic cells was observed. To conduct clinical and experimental studies, three groups of animals were formed. As a result, it was found out that subclinical mastitis of cows is accompanied by a change in the immunobiological reactivity. Purulent-catarhal mastitis in cows was manifested by significant changes in the parameters of nonspecific immunological reactivity. In the peripheral blood of cows with subclinical mastitis, the number of reactive microphages increased sharply (P < 0.001). In parallel with this, 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 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-catarhal mastitis of cows undergo significant changes in systemic immunity. In the pathophysiological model of subclinical and purulent-catarhal 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, local immunity, immunocompetent cells, mastitis, 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).

In the 70s of the last century a new scientific direction — reproductive immunology — was created that integrated fundamental and applied immunological studies of related branches of biology and medicine. A new scientific school of animal reproduction immunology, headed by the doctor of biological sciences, professor, correspondent member of the National Academy of Sciences of Ukraine V.A. Yablonsky, was formed on the territory of Ukraine. Modern researches of this school have greatly expanded the knowledge about the role of immune mechanisms associated with the reproductive capacity of animals. Nowadays, mammalian immunology laboratories have been conducting research on immunology of lactation and improving methods for assessing the immune status of animals (Rainard, 2017; Yablonskyi and Zhelavskyi, 2018).
The immune status of the cows was determined using the developed immunocard (Yablonskyi and Zhelavskyi, 2008; 2010; 2014), which included a step-by-step determination of indices of nonspecific resistance and specific immunobiological reactivity. Immunological studies examined the cellular, humoral, and non-specific localized immune secretion of the mammary gland. Phagocytic activity of leukocytes (FA) was determined in reaction with inert polystyrene particles of latex, phagocytic number (FN), phagocytic index (FI), and phagocytic capacity (FC).

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, MAMW); other immunobiological parameters (LGI, LII, RNN) (Yablonskyi and Zhelavskyi, 2008; 2010).

Determination of the cytochemical reactivity of phagocytes in the secretion of the mammary gland was carried out according to our patented method (Patent of Ukraine for Utility Model No. 73635, MPK7A61V 10/02 (2006.01), Method for evaluating the antimicrobial reactivity of the cow’s breast secretion secretory neutrophils. All studies were conducted in accordance with the Law of Ukraine “On Protection of Animals from Cruel Treatment” (No. 3447-IV of February 21, 2006) and the current requirements of the European Commission for treating vertebrate animals and protecting them from thirst, hunger, malnutrition, discomfort, fear, pain and illnesses.

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

Results and discussion

As it is known in plasma and serum of blood mammals, regardless of antigenic stimulation, there is always a complement that is essentially a whole system of 11 different protein components. Under physiological conditions in the secretion of the mammary gland there are low concentrations of complement. In the secretion of the breast of the cows, the concentration of the component of complement C3 is only 2.5% of its content in the peripheral blood. In the process of inflammation, the exudative reaction is accompanied by the activation of the C3b/C3bi components. The value of C5a, which stimulates chemotaxis and migration of neutrophils in the inflammatory site, has been carefully studied. In serological studies in vitro and in vivo, the development of a mastitis for the participation of Escherichia coli or S. uberis, accompanied by an intensive leukocyte response, was confirmed (Pang et al., 2017; Günther et al., 2017).
The expressed antimicrobial properties also have lactoferrin, which in its structure is Ferum-binding glycoprotein, which is contained in large numbers in the secondary granules of neutrophil granulocytes. According to, the concentration of lactoferrin in the secretion of the mammary gland ranges from 20–200 mg/ml and significantly increases during the inflammation. The formation of free radicals thus causes the destruction of membrane structures and the death of microorganisms. Experimental studies have also proven that lactoferrin stimulates the activity of lactoperoxidase. The susceptibility to E. coli and Staphylococcus aureus was shown, that microbial strains of Streptococcus agalactiae, Streptococcus dysgalactiae and Streptococcus uberis, which have lactoferrin binding proteins on their surface, reduce the bactericidal effect of lactoferrin (Kempf et al., 2016; Delfani et al., 2017; Günther et al., 2017). Also the data on immunomodulatory and neutralizing properties of lactoferrin were published. In particular, it was proved that the introduction of lactoferrin in the beginning of the inflammatory process in the mammary gland causes the extinction of the inflammatory reaction, in addition, it is able to bind to the lipopolysaccharides, while blocking their toxicogenic effect. The composition of Ferum-containing glycoproteins also includes transferrin. In contrast to milk from mice and rabbits, the secret of the cows’ mammary gland contains transferrin in low concentrations: from 1 mg/ml in colostrum and to 0.02–0.04 mg/ml in milk. Transferrin is delivered to the mammary gland from the blood, where its serum concentration is 4–5 mg/ml. In the acute mastitis (E. coli), the content of transferrin may reach 1 mg/ml, which is probably related to the Ferrum metabolism. Significant importance in the formation of antimicrobial protection plays lysozyme, the mechanism of action of which is based on the enzymatic hydrolysis of N-acetylmuramic bonds in the peptidoglycine complex (N-acetylmurmonic acid and N-acetylglucosamine) of the microorganism wall. The secretion of the mammary gland contains a small amount of lysozyme (0.13 μg/ml), which comes from the blood serum, and is excreted by macrophages (Blum et al., 2015; Cao et al., 2018).

Serial clinical and experimental studies have traced the main stages of ontogenesis of cellular immunity of the mammary gland of cows. The total number of somatic cells in the secretion of the mammary gland of the primates varied from 180.78 ± 11.84 to 957.03 ± 12.03 thousand/ml (P < 0.001), which depended on the period of functioning of the mammary gland: in the colostrum, the secrecy prevailed (56.00 ± 1.90%) neutrophilic granulocytes, in the middle lactation period (3–5th month) the proportion of epithelial cells increased (from 29.51 ± 2.17 to 49.59 ± 1.94%, P <0.001), during the launch period, the population of polymorphonuclear neutrophil granulocytes practically recovered to the baseline level (60.51 ± 1.28%, P < 0.001) and then increased (to 66.07 ± 1.61%, P < 0.001) during the dry period (Zhelavskyi, 2014).

**Fig. 1.** Manifestation of cytochemical reactivity of neutrophil granulocytes (x 2000) secretion of the mammary gland of cows in reaction to IL. Atraction (a) and phagoms formation (b) with microbial strain *Micrococcus lisodeikticus*

The cytochemical reactivity of intracellular lysozyme (ILL +) phagocytic cells in the secretion of the mammary gland (Figure 1: A, B) of the primapar from the beginning of lactogenesis and in the middle period of lactation (3–5th month) was at a constant level (34.25 ± 0.81 and 37.33 ± 0.83, respectively).

However, at the end of lactation, during the onset and dry, with the development of involutional processes in the mammary gland of the primates, a sharp decrease (to 26.55 ± 0.75%, P < 0.01) of the cytochemical reactivity of IL + phagocytic cells was observed (Zhelavskyi, 2010).

There is experimental evidence that lysozyme may exhibit antimicrobial activity as an independent microbial substance, and is associated with lactoferrin and opsonating antibodies (Lombardini et al., 2017).

Modern literature on the antimicrobial effects of lactoperoxidase, an enzyme protein of milk, which is excreted by neutrophilic granulocytes, should be taken into account. According to the latest data, Lactoperoxidase exhibits antimicrobial effect involving hydrogen peroxide (H₂O₂) and thiocyanate (SCN⁻), catalyzing the formation of bactericidal mediators (SCN)₂, HOSN⁻, HO₂SCN, HO₂SN, and endonuclease metabolites ³SO₂⁻. Hypothyocyanide (OSCN⁻) inhibits NADN-dependent glycolaldehyde-3-phosphate dehydrogenase, which is the cause of glycolysis in bacterial cells (Blum et al., 2015; Law et al., 2017; Herry et al., 2017; Rainard, 2017).

A similar trend was observed in the phagosomal activity of lysosomal cationic proteins (LCP). The growth of LCP activity in phagocytic cytoplasm during dry
The greatest reactivity of phagocytes was shown in the beginning of lacto-genesis of primates in the reaction to lysosomal cationic proteins (neutrophil activation index, IAN 0.92 ± 0.08), which acquired its maximum manifestation during the 3–5th month of lactation (1.36 ± 0.03, P < 0.001), – during the period of the largest functional load of the mammary gland, and in the future (start, dry weight) gradually decreased (P < 0.01).

During colostrum, in the secretion of the breast of the firstborn, the amount of myeloperoxidase (MPO) – positive cells was 51.48 ± 0.57%, IAN 0.87 ± 0.03, and the CLI was 1.5 ± 0.03%. During the 3–5th month of lactation, the total number of MPOs + phagocytic cells gradually decreased (42.07 ± 0.61%, P < 0.001) with a certain increase in IAN (1.03 ± 0.07, P < 0.001) and cytological index (1.82 ± 0.07%, P < 0.001).

During the lactation, in the secret of the pruritic loss, there was also pronounced activation of the antimicrobial potential of phagocytes in the NBT-test. Thus, at the beginning of lactation, the number of formazanopositive neutrophil granulocytes in their colostrum was 32.96 ± 0.93%, in the middle – gradually decreased to 12.8 ± 1.01% (P < 0.01), and at the end of lactation, the inverse wavelength increased: 16.67 ± 0.55% (P < 0.01) in start and 32.00 ± 0.73% (P < 0.001) in dry condition.

Our studies have proven that local breast immunity is ontogenetically formed, and the complete formation of cellular factors of immune defense in the womb takes place in several stages. Experimental studies showed a significant increase in the activity of LCP in the phagocyte cytoplasm in the dry period (62.62 ± 0.56 versus 53.81 ± 0.78%, P < 0.01 in the colostrum period) and in the middle period (3–5th month) secretion milk against the background of a decrease in the percentage of the cytological index (CLI, P < 0.001) and the total cytochemical reactivity index in the second and third period of the functioning of the mammary gland (Zhelavskyi, 2014; 2015; Zhelavskyi and Shunin, 2017).

The greatest reactivity of phagocytes was shown in the beginning of lacto-genesis of primates in the reaction to lysosomal cationic proteins (neutrophil activation index, IAN 0.92 ± 0.08), which acquired its maximum manifestation during the 3–5th month of lactation (1.36 ± 0.03, P < 0.001), – during the period of the largest functional load of the mammary gland, and in the future (start, dry weight) gradually decreased (P < 0.01).

Thus, in our serial studies, it was found that in the colostrum period the number of myeloperoxidase (MPO) – positive cells was 51.48 ± 0.57%, IAN 0.87 ± 0.03, and the CI of 1.5 ± 0.03%. During the 3–5th month of lactation, the total number of MPOs + phagocytic cells gradually decreased (42.07 ± 0.61%, P < 0.001) with a certain increase in IAN (1.03 ± 0.07, P < 0.001) and cytological index (1.82 ± 0.07%, P < 0.001).

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; 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 (LGR, 0.73 ± 0.07, P < 0.01), which was more aggravated by the development of a purulent-catarhal inflammatory process (0.61 ± 0.03, P < 0.01). Along with this, the leucocyte intoxication index (LII) was changed – the marker of the depth of endogenous intoxication by metabolites of inflammation (microbial toxins, cellular elements, peptides, etc.).

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-catarhal 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 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.

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) (Yablonskyi and Zhelavskyi, 2014). The total index of cytochemical potential in subclinical mastitis 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 (Yablonskyi and Zhelavskyi, 2010).

In the biometric processing of data sets, it was found that in subclinical mastitis, there is a direct correlation (r = 0.82) between LASB and the neutrophil degranulation index (NDI), which convincingly proves that in the pathogenesis of subclinical mastitis, neutrophil 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).

As 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, 2010; 2014). Usually, autoantigenic reactions in the body are controlled by immunocompetent cells, which forms the basis of immune homeostasis (Zhelavskyi, 2015; 2018). 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, 2010; 2011; Ceniti, 2017). Excessive formation and imbalance of CIC and MAMW (SM) often leads to suppression of the functional state of immunocompetent cells and the development of immunocomplex inflammation (Zhelavskyi, 2008; 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 circu-
iating 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 2).

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

Conclusions

In the process of ontogenetic development of the breast of the primates in parallel with the formation of secretory function of the mammary gland there is a gradual formation of cellular factors of its local defense. Oxygen-independent and Oxygen-dependent factors of phagocytic protection of the breast of the firstborn are not yet sufficiently formed, their activation begins with the colostrum period and undergoes a permanent oscillation throughout the lactation period.

Clinical and experimental studies have shown that subclinical and purulent-catarhal mastitis of cows undergo significant changes in systemic immunity. In the pathophysiological model of subclinical and purulent-catarhal 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-catarhal 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.

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

With purulent-catarhal mastitis, there was a sharp increase in the CIC of medium-molecular CICm (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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