POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 35(3): 363–378, Y. 2020

EXTRACELLULAR ANTIBACTERIAL DEFENSE MECHANISMS OF NEUTROPHIL GRANULOCYTES AND THEIR ROLE IN PATHOGENESIS OF PYOMETRA (CASES) IN CATS

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Key words: cats, pyometra, local immunity, phagocytes, neutrophil extracellular traps.

Abstract

Pyometra in cats is the most common reproductive pathology with septic inflammation in uterus, accompanied by a cascade of immunological responses and changes in local homeostasis. The aim of the research is to study the extracellular protective mechanisms of neutrophil granulocytes in the course of immunological response during the development of pyometra in cats. The observed acute inflammatory reaction in the infected organism was accompanied by the active migration of phagocytes to the pathological to the site of inflammation. The pathology was demonstrated by the active growth of cytochemical reactivity of the Oxygen-dependent antimicrobial potential of the neutrophils in genital mucosa and the initiation of extracellular protective trap formation mechanism. Cytological markers of phagocytic cells detected in the pathogenesis of the pyometra should be taken into account during the diagnosis of the pyometra, prognosis of the course of this reproductive pathology and analysis of the adequacy of therapy.

Introduction

Pyometra is common reproductive pathology in cats and is characterized by cystic endometrial hyperplasia and septic inflammation which develops secondary to hormone-dependent alteration of endometrium

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(Dmirel and Acar 2012, Hollinshead and Krekeler 2016, Zhelavskyi and Shunin 2017). Pathogenesis of the pyometra is complex and can be described by the development of dysfunctions in all organs and systems (Chen et al. 2012, Graham and Taylor 2012, Hagman 2018). Despite this, immune defense mechanisms are of crucial importance in pathogenesis of this condition (Maciel et al. 2014, Grayson and Kaplan 2015, Jursza et al. 2015).

Neutrophils are a population of immunocompetent cells that have a number of membrane receptors on their surface and are able to respond to changes in homeostasis (Khan et al. 2011, Kaplan and Radic 2013, Jursza-Piotrowska and Siemieniuch 2016). Neutrophil granulocytes are the first-responders during inflammation (Caudrillier et al. 2012, Chen et al. 2014), which first migrate into the pathological process area and realize their phagocytic function (Wira et al. 2005, Gould et al. 2014).

In infected tissues, neutrophilic granulocytes destroy microorganisms by involving cellular and extracellular mechanisms of antimicrobial defense. The study of the structure, physiology of neutrophilic granulocytes, the their biochemical composition, and the mechanisms of interaction is very relevant (Akong-Moore et al. 2012, Gray et al. 2013, Chen et al. 2014). Phagocytes is has by the active role that they play in maintaining the homeostasis of the body (Parker al. 2012, Kaplan and Radic 2013). The main function of neutrophilic granulocytes is phagocytosis. The objects of phagocytosis are usually biological agents having a corpuscular structure (bacterial and fungal pathogens, protozoan cells, their own damaged cells and their decay products). Neutrophils absorb and digest captured microorganisms using Oxygen-dependent and Oxygen-independent mechanisms, which leads to their elimination (Wira et al. 2005, Zhelavskyi and Shunin 2017, Vilhena et al. 2018).

The neutrophil cytoplasm contains three main types of granules – primary (azurophilic), secondary (specific) and tertiary. Primary azurophilic granules include myeloperoxidase (MPO), which is necessary for the enzymatic conversion of H_2O_2 to HOCl. They also contain harvested neutrophil elastase and defensin proteins. Defensins are embedded in the microbial membrane with a violation of its integrity. They can also destroy the DNA of bacteria (Caudrillier et al. 2012, Metzler et al. 2014, Jursza et al. 2015). Secondary granules contain lysozyme, lactoferrin, gelatinase and metalloproteases. Their membranes also contain up to 95% cytochrome B558, a component of the NADPH oxidase enzyme (nicotinamide adenine dinucleotide phosphate oxidase). The main enzyme of tertiary granules is gelatinase (Khan et al. 2011, Kahlenberg et al. 2013, Kenny et al. 2017).

Neutrophils have the ability to respond even to minor environmental changes using an extensive array of membrane receptors from different families (Futosi et al. 2013, Chu et al. 2016). On their membranes are presented, for example, Toll-like receptors (TLR), Fc receptors for immunoglobulins of various classes, primarily IgG, receptors for complement components (C3b and others), which ensures efficient opsonization of phagocytosis objects (Jaillon et al. 2016, Nowak et al. 2019). In infected tissues, neutrophilic granulocytes collide with microorganisms and are activated, absorbing the pathogen in vacuoles (phagosomes). Further, neutrophil granules merge with the phagosome, forming phagolysosomes into which antimicrobial peptides and enzymes fall. Moreover, in the phagolysosome, microorganisms are exposed to high concentrations of reactive oxygen species (ROS) (Kambas et al. 2012, Diana et al. 2013, Perez-de-Puig et al. 2015). For a long time, neutrophils were considered only as nonspecific effector cells of innate immunity, realizing all of the above functions. After completing his biological program, the neutrophil dies. Apoptosis after phagocytosis or possible necrosis under the influence of the pathogenicity factor of pathogens was considered the most probable outcome of differentiated neutrophils (Caudrillier et al. 2012, Kidd et al. 2013, Harbort et al. 2015).

In 2003 Volker Brinkmann (Max Planck Institute For Infection Biology, Germany) a new mechanism for the antimicrobial action of neutrophils has been described. It turned out that neutrophilic granulocytes after activation eject network-like structures into the extracellular space (Knight and Kaplan 2012). Space, which include DNA, histones, as well as various proteins and granule enzymes, such as elastase and myeloperoxidase. These structures were called “neutrophilic extracellular traps” (Neutrophil Extracellular Traps, NETs) (Harbort et al. 2015, Amulic et al. 2017). Initially, the purpose of this phenomenon was unclear. Nevertheless, it was immediately suggested that network-like structures isolate and destroy gram-positive and gram-negative bacteria, fungal pathogens. It should be noted that processes resembling the formation of NETs also mentioned in earlier sources.

The integrity of the outer membrane of granulocytes activated in this way does not affect the destruction of the inner membranes that allow mixing of the intracellular components of the neutrophil. This process directly depends on the formation of ROS. Most likely, this is one of the first descriptions of the formation of NETs (De meyer et al. 2012, Kahlenberg et al. 2013). The formation of extracellular traps (or NETosis, “netosis”) is another variant of the fate of a neutrophilic white blood cell (Chowdhury et al. 2014, Grayson and Kaplan 2016).

It is known that the intensity of the inflammatory reaction depends largely on the cascade of immunological *responses* in which the cellular mechanisms of protection are involved (Khan et al. 2011, Parker et al. 2012, Zhelavskyi and Shunin 2017). In view of this, neutrophil granulocytes play an important role in maintaining homeostasis. At the same time, the cellular factors of local immunity of reproductive organs of cats are still not sufficiently studied, which makes for the necessity of a detailed research of the mechanisms of antimicrobial protection both at normal state and with the pathogenesis of the pyometra. Consequently, the functional capacity of phagocytes is an important indicator value. Its exploration is promising for the detection of informative cytological markers of inflammation and will be useful for improving the methods of cat pyometra diagnosis.

The aim of the research is to study the extracellular protective mechanisms of neutrophil granulocytes in realization of local immunity during the development of pyometra in cats.

Materials and Methods

Animals' criteria. Clinical and experimental studies were a performed in the veterinary clinic and in the Specialized Laboratory of Immunology of Reproduction Animals (State Agrarian and Engineering University in Podilya, Kamianets-Podilskyi, Ukraine). For the experimental part of the work, control groups (healthy, n = 17) and experimental (with an open form of the pyometra n = 17) of cats were formed.

The diagnosis of the pyometra was based on interview (history), clinical symptoms, laboratory (cytological, microbiological, haematological, immunological) and ultrasound examination (Toshiba Core Vision Pro, Japan, linear transducer 8-MHz).

Blood collection and analyses. Blood samples for haematological analysis were tested in the Specialized Laboratory of Immunology of Reproduction Animals, Department of Veterinary Medicine, State Agrarian and Engineering University in Podilya, Ukraine. The above mentioned analyses were performed using routine methods, parameters (RT-7600 VET) included haemoglobin (Hb), haematocrit, red blood cell count (RBC), white blood cell count (WBC), differential count of WBC including total count (BN) and percentage band neutrophils (PBN).

Determination of antimicrobial potential of neutrophils in reaction with nitro blue tetrazolium (NBT-test). Using a special brush for cytology pre-wetted with 15 M phosphate buffer NeoGalin18 (15 M NaH_2PO_4 + $2H_2O$ (11.8 g) + KH_2PO_4 (68.0 g) + C_6H2O_6 (10.0 g), pH 7.2) samples from dorsal parts of the vagina, cervix and the exudate were obtained from the uterus, which were applied to the microscope slide. 0.05 ml of 0.15% solution of nitro blue tetrazolium (produced by Renal®, UK; phosphate buffer (pH 7.2) were added to the cell mixture. Subsequently, the microslides were incubated for 30 minutes in a wet chamber of the thermostat (37°C). After incubation, smears stained with methanol and stained with 0.1% phosphate buffered neutral red (pH 7.2) were prepared (ZHELAVSKYI 2017).

Assessment and accounting of metabolic reactions of phagocytes (determination of the percentage of reactive neutrophils) were carried out microscopically (2500 x magnification). Reactive (NBT reactive) neutrophil granulocytes were visualized by the presence of dark-brown inclusions in the cytoplasm in the cytoplasm in the form of fine diffuse grains. The intensity of the "respiratory burst" (the percentage of active microphages that showed reactivity) was determined using IV stages. A zero (0) degree was characterized by a complete absence of granulomas of formazan in phagocytic cytoplasm cells. Such microphages were classified as intact. The first (I) degree was manifested by the formation of single granules in phagocytic cells. The second (II) – in the presence of inclusions occupying almost 1/3 of the cytoplasm. To the third (III) degree belonged cells in which cytoplasmic inclusions occupied 2/3 of the cell area. The highest IV level of metabolic reactivity of phagocytes was expressed by the formation of intense, well-expressed granules of diformasan, which were visualized throughout the cytoplasm, including the nucleus of microphage cells (KENNY et al. 2017).

While determining the degree of reactivity and interpretation of cytochemical indicators, following indices were calculated: the cytological index (CLI [%]; index of activation of neutrophils (IAN in standard units of measurement (c.u.); the index of migration activity of neutrophils (IMN in standard units of measurement (c.u.) and the ratio of phagocytes to epithelial cells (Fag/Epithel) (ZHELAVSKYI 2017).

Determining the ability of neutrophils to form NETs (Neutrophil extracellular traps). The diagnostic material was obtained from the vaginal mucosa taken with a cytologic brush pre-moistened with 15 M phosphate buffer NeoGalin18 (pH 7.2). Then a smear was prepared. After drying at room temperature (20°C), the microslide was fixed with methanol and stained with 1% phosphate buffered saline solution (pH 7.2) at exposure for 2–3 min. After that, the *microslide* was rinsed with phosphate buffer and stained with a dye-fixative eosin methylene blue for May-Grünwald. Estimation of neutrophils from NETs [%] was carried out microscopically – 2500x magnification (ZHELAVSKI 2017).

Results

The cat's pyometra is usually observed at the age from 3 to 10 years. During the entire observation period (2014–2019), the disease was registered in 382 cats. The disease was manifested in animals from 3 years. The open form of the pyometra was observed 14.1±0.72%. The largest risk group was the 5 years old animals (16.7±0.47 %) and the incidence of disease continued to decrease gradually.

Signs of the illness were diagnosed in the luteal phase. Breed predisposition detailed study, the animal's predisposition to the development of the pyometra was established (Table 1). Most often reproductive pathology was manifested in *Persian breed* (24.2±0.62%), *Turkish Angora* (20.7±0.57%) and Siamese (19.3±0.52%).

Table 1

Breed predisposition of cats to the pyometra (Mean±SD)

Breed	Frequency of pyometra [%]
Turkish Angora	20.7±0.57
European group	7.8±0.27
Brittan	15.8±0.37
Persian	24.2±0.62
Siamese	19.3±0.52
Domestic cat	12.2±0.42

It was found that in open form of the pyometra the main symptoms of the disease in animals include lethary/depresssion, fever, tachycardia, dysuria, abdominal distension. The most clinical symptoms were haemopurulent vulvar discharge, hyporexia/anorexia, vomiting and weight loss. Ultrasonography has revealed the presence of fluid within the lumen of the uterus. The uterine wall often appeared thickened with irregular edges and small hypoechoic areas consistent with cystic changes to the endometrial glands.

In a haematological examination, an increase in the number of leukocytes ($33.01\pm1.27 \cdot 10^9$/L, $P < 0.01$) and signs of severe neutrophilia (75.88±0.99%, $P < 0.01$) were observed. Moreover, red cells amount ($5.17\pm0.25 \cdot 10^{12}$/L, $P < 0.05$), haemoglobin and haematocrit lever decreased as well (Table 2). Acute inflammatory reaction was accompanied by active migration of phagocytes into the area of the pathological process (IMN, Figure 1). In microslides taken from the vaginal mucosa, an increase in the number of neutrophilic granulocytes was observed (26.23±1.03%, $P < 0.01$, Table 3). The inflammatory response was accompanied by a cell imbalance with an increase in number of phagocytes (Phag/Epithel 1.14±0.04, $P < 0.05$) and IAN (0.34±0.01, $P < 0.01$, Figure 2).

Table 2

Hematological indices of cats with a pyometra (Mean ± SD)

Variables	Healthy feline (*n* = 17)	Hospitalized feline (*n* = 17)
WBC [$\cdot 10^9$/L]	17.05±0.74	33.01±1.27**
RBC [$\cdot 10^{12}$/L]	7.21±0.42	5.17±0.25*
Hemoglobin [mmol/L]	11.72±0.53	7.21±0.27**
Hematocrit [L/L]	23.15±0.52	38.17±0.67*
Neutrophils [%]	46.01±0.86	75.88±0.99**

n – number; *$P < 0.05$; **$P < 0.01$; WBC – white blood cells; RBC – red blood cells

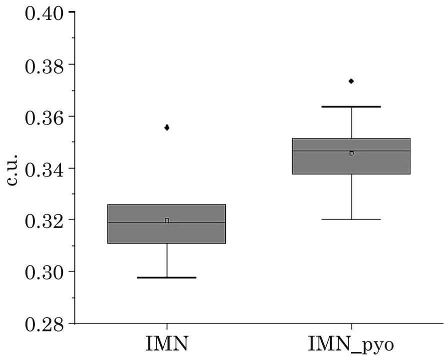

Fig. 1. Migratory activity of neutrophil granulocytes (Mean ±SD): IMN – index of migratory activity of neutrophils; IMN_pyo – index of migratory activity of neutrophils, patients with cats' pyometra; c.u. – conditional units of measurement

Table 3

Cytological indices of the mucous membrane of the vagina of cats with a pyometra (Mean ± SD)

Variables	Healthy cats (*n* = 17)	Hospitalized cats (*n* = 17)
Neutrophils mucosa [%]	14.70±0.68	26.23±1.03**
Phagocyte/Epithelial cell	0.17±0.09	1.14±0.04*

n – number; *$P < 0.05$; **$P < 0.01$

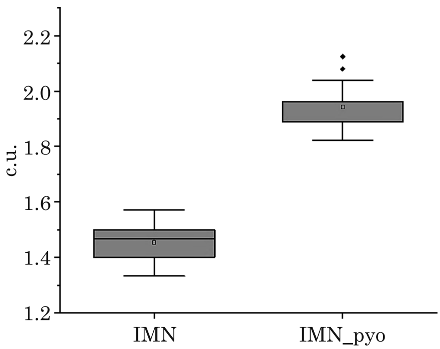

Fig. 2. Activation of phagocytic activity during pyometra (Mean ± SD): IAN – neutrophil activation index; IAN_pyo – index of activation of neutrophils, patients with cats' pyometra; c.u. – conditional units of measurement

The pathological process was manifested by the growth of cytochemical reactivity neutrophils (NBT 50.88±0.85%, $P < 0.01$) with the highest activity of III (18.41±0.50%, $P < 0.01$) and IV (14.29±0.77%, $P < 0.01$). In this case, the CLI increased significantly to 1.43±0.02, $P < 0.01$, Table 4).

Table 4

Indicators of antimicrobial activity of Oxygen-dependent mechanisms of protection of healthy cats and hospitalized whith pyometra (Mean ± SD)

Variables	NBT-test [%]	Cytochemical reactivity [%]					
		0 stage	I stage	II stage	III stage	IV stage	CLI
Healthy cats (n = 17)	21.35±0.86	78.64±0.86	10.41±0.50	7.64±0.49	2.29±0.46	1.01±0.35	0.36±0.02
Hospitalized cats (n = 17)	50.88±0.85**	77.94±0.76	2.64±0.49**	18.41±0.50**	15.52±0.51**	14.29±0.77**	1.43±0.02**

n – number; NBT-test – cytochemical reactivity of neutrophils in reaction with nitro blue tetrazolium; 0–IV stage – step intensity of cytochemical reactivity of neutrophils; CLI – cytological index; $^{**}P < 0.01$

The number of neutrophilic granulocytes NETs (61.94±0.89%, $P < 0.01$; Table 5) increased on the mucous membranes.

Table 5

NETs activity of neutrophils of the mucous membrane of cats' genitals with pyometra (Mean±SD)

Variables	Healthy cats (n = 17)	Hospitalized cats (n = 17)
NETs [%]	27.05±0.82	61.94±0.89**

n − number, NETs − neutrophil extracellular traps; $^{**}P < 0.01$

Endometrial inflammation was also manifested by an exudative reaction with active involvement of phagocytic cells in the pathogenic pathology area (NETs, Figure 3 and Figure 4).

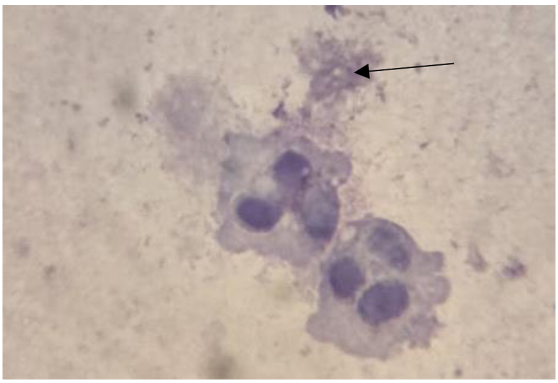

Fig. 3. Formation of protective traps NETs (black arrow) by microphages of the uterine mucosa (2500x magnification with Malachite green and May-Grünwald staining)

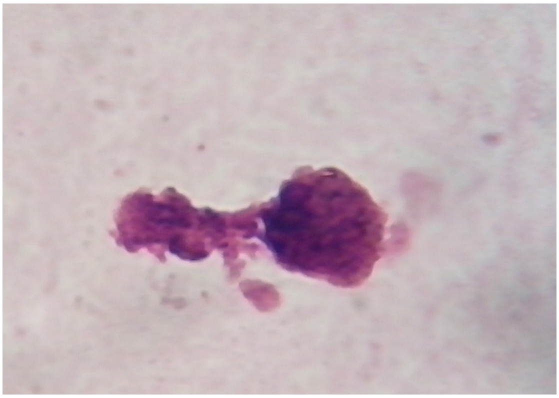

Fig. 4. Neutrophil granulocyte with NETs (2500x magnification with May-Grünwald staining)

Discussion

Scientists from many countries around the world are focused on the study of local factors in the protection of reproductive organs of animals (WIRA et al. 2005, MACIEL et al. 2014, JURSZA et al. 2014). More attention is paid to cellular mechanisms of protection as one of the components of immune homeostasis (RISSO et al. 2014, LI and TABLIN 2018). There are isolated reports on the state of phagocytic protection of the mucous membranes of the reproductive organs of animals in different periods of the oestral cycle and in the development of the pyometra (REBORDÃO et al. 2017, PAPAYANNOPOULOS 2018).

Neutrophil products secreted into the trap space have selective bactericidal properties. They inhibit the growth of pathogenic and conditionally pathogenic microorganisms, but have little effect on non-pathogenic microorganisms, in particular lactic or bifidobacteria (JAILLON et al. 2016). This may be due to differences in the mechanisms of neutrophil stimulation by pathogenic and non-pathogenic pathogens. It was previously found that intracellular neutrophil killer systems are more activated by *E. coli* and *S. aureus* than by *Lactobacterium spp.* and *Bifidobacterium spp.* (MANTOVANI et al. 2011, JAILLON et al. 2016). In addition, cells secrete a different set of antimicrobial mediators upon neutrophil activation through separate groups of receptor molecules, such as image-recognition receptors. Bacterial opsonization (eg, interaction of specific IgG with the envelope *S. aureus*) also stimulates the production of NETs by acting through Fc receptors of leukocytes (KRAMER et al. 2017). Obviously, along with microbial stimulation, trap formation is also regulated by numerous external factors and signaling. Pro-inflammatory agents such as interleukin-8, lipopolysaccharide (LPS) or phorbolmyristate acetate are provoked by the formation of traps. In turn, the NADPH oxidase inhibitor diphenylene iodone prevents the formation of NETs. Separately, IL-8 and LPS release traps less efficiently than bacteria. Optimal leukocyte NETs formation requires activation through several receptors (FUTOSI et al. 2013, JAILLON et al. 2016). On the other hand, it turned out that neutrophils of whole blood and its leukocyte suspension did not normally form spontaneous NETs, despite the periodic increase in the concentration of activators in serum (KHAN et al. 2011, DE MEYER et al. 2012, HAZELDINE et al. 2014).

Moreover, the process of death of granulocyte significantly differs from apoptosis and necrosis, which were studied previously (GRAHAM and TAYLOR 2012). Studies have shown that network formation is a controlled process, and not an accidental release of granules and nuclear contents of a cell, as during necrosis or apoptosis. It has been established that networks can form as an alternative to phagocytosis. Compared to apoptosis and necrosis, the most important morphological differences in netosis are decay of the nuclear membrane and mixing of nuclear and cytoplasmic material, loss of the inner membrane and disappearance of cytoplasmic organelles. Neutrophil apoptosis is a strictly regulated response that seeks to prevent cell contents from entering the intercellular space. NETosis, in contrast, is directed to the controlled release of intracellular granulocyte components. This process is also subject to strict regulation. Unlike apoptosis, it is stimulated by ROS, but does not depend on caspases. In this case, DNA fragmentation does not occur, but nuclear membrane destruction is observed (KNIGHT et al. 2012, BRODZKI et al. 2015, HARBORT et al. 2015, LI et al. 2018).

During neutrophil activation, a cellular signaling system is induced, including phosphatidyl-inositol-3-kinase and serine-threonine kinase (STK), which is responsible for protein synthesis, microtubule function, and neutrophil autophagy. This system takes part in the disintegration and rupture of the cell membrane during "netosis" (CHOWDHURY et al. 2014, YAN et al. 2015). After activation of the cytoskeleton, the cell contracts until the outer membrane ruptures. A highly active mixture, once in the extracellular space, forms a peculiar three-dimensional network, a "trap", into which bacteria enter. The neutrophil dies. This oxygen-dependent cell death was called the term "NETosis" (FUCHS et al. 2012, MCINTURFF et al. 2012, GOULD et al. 2014). This suggests that in the bloodstream of healthy animals, the formation of NETs should not occur. Many authors have shown that the formation of NETs in the bloodstream mechanically disrupts blood circulation in the tissues and organs (REBORDÃO et al. 2017, PAPAYANNOPOULOS 2018). Blood inhibitory factors have been found to have a humoral nature. Autologous serum and blood plasma inhibit extracellular DNA release by neutrophils isolated from peripheral blood. Thus, in the systemic blood flow, in the absence of inflammation, the formation of NETs is suppressed (MACIEL et al. 2014, JEFFERY et al. 2016). Neutrophils of patients with chronic granulomatous disease are known to be unable to generate ROS due to a deficiency of the NADPH oxidase enzyme. In turn, the neutrophils of these patients were unable to form NETs. However, at least in part, the glucose oxidase enzyme compensated for the functional failure of NADPH oxidase to produce hydrogen peroxide (YAN et al. 2015). However, under the influence of glucose oxidase, the formation of NETs significantly increased. In connection with all the above data, it can be concluded that, on the one hand, NETs function as an effective antimicrobial barrier, on the other hand – their excess leads to the development of inflammatory processes and to hemodynamic disorders in case of deficiency of counteracting regulatory mechanisms. Neutrophils are, above all, tissue cells involved in inflammatory and antimicrobial reactions. They also function actively in the mucous membrane (CAUDRILLIER et al. 2012, GOULD et al. 2014). It is obvious that disorders of mucosal immunity contribute to the recurrent course and chronicity of local inflammatory processes (GRAY et al. 2013).

According to some authors, the formation of NETs is a mechanism of protection that acts in the tissues and on the surface of the mucous membranes, and is especially important in the mucosal anti-infective protection (JEFFERY et al. 2016). Neutrophils leaving the tissues and leaving the mucous membranes can participate in the antimicrobial protection and in the regulation of the microbiota of the respective biotopes, secreting biocidal products (JURSZA et al. 2015, ZHELAVSKYI 2017). In addition, the aggressive factors of neutrophil granules in the trap formed are linked by DNA strands. In the case of colonization of tissues or mucous membranes by representatives of normal microflora, trap components are not capable of causing the development of inflammatory responses (MANTOVANI et al. 2011, ZHELAVSKYI and SHUNIN 2017, Li et al. 2018).

In the presented study the state of phagocytic protection of mucous membranes of genitalia of cats during development of a pyometra is considered. In our studies, it has been found that in the pathogenesis of the pyometra there is a cascade of immune reactions. Neutrophilic granulocytes are actively migrating from the peripheral blood to the zone of the pathological process. The launch of phagocytic reaction takes place. Activated neutrophils carry out an active attack of microorganisms and involve extracellular mechanisms of protection (PARKER et al. 2012, HAZELDINE et al. 2014, JURSZA et al. 2015).

In the extracellular space, phagocytes excrete a number of antimicrobials, including reactive oxygen species (ROS) (De Meyer et al. 2012, Zhelavskyi and Shunin 2017). Our study found that the total number of activated neutrophils of mucosal surfaces of reproductive organs can excite generation of ROS. At the same time, the inflammatory reaction was accompanied by activation of the formation of NETs. This, in our opinion, is induced by pathogenic strains of microorganisms that have penetrated the zone of the pathological process.

The process of creating NETs begins with the activation of neutrophil. The launch of the membrane-binding multimolecular enzyme complex NADPH oxidase takes place. "Respiratory burst" is being activated. Formulated by ROS (Hazeldine et al. 2014, Luo et al. 2014, Jeffery et al. 2016) that induce activation of enzyme systems of phagocyte (elastase and Protein arginine deiminase 4 (PAD-4) (Wira et al. 2005, Papayannopoulos 2018). There is a conversion of arginine and methyl arginine to cerulin in the histone proteins of the nucleus. The consequence is the decomposition of chromatine and the release of DNA (Kaplan and Radic 2012, Luo et al. 2014, Martinod and Wagner 2014).

In the process of activating neutrophils, a cellular signaling system, including phosphatidylinositol-3-kinase and sertronicin kinase (STK), which is responsible for protein synthesis, microtubule function and neutrophil autophagy, is induced. This system participates in the disintegration and rupture of the cell membrane during the "NETosis" (Parker et al. 2012, Mcinturff et al. 2012, Papayannopoulos 2018).

After the activation of the cytoskeleton, the formation of a volumetric grid, a "trap", occurs in which bacteria enter. Neutrophil dies at the same time. In the course of the formation of NETs, in conjunction with decondensated chromatin (DNA and histones), proteases and antimicrobial peptides, that are contained in neutrophil granules, are released. These indicators are important markers of inflammation (Jursza et al. 2015, Jeffery et al. 2016, Zhelavskyi 2018). Recent studies have proven the importance of cellular protective factors for local immunity of the animal's genital organs. studies have shown that neutrophil granulocytes are the primary messengers of the inflammatory process. Microphages actively migrate from the peripheral bloodstream. Neutrophil granulocytes show active protection through the realization of extracellular protection factors. Changes in cytochemical markers can be taken into account for the diagnosis of pyometra (subclinical manifestations, closed pathology). And as well can be taken into at the prognosis of this reproductive pathology (Zhelavskyi 2019). On the other hand, it can be used to analyze immunological shifts and develop adequate therapy.

Conclusion

Cat's pyometra is a widely spread reproductive disease that occurs due to the changes in endocrine regulation and immune homeostasis. Cascade of disturbances of mechanisms in local immune protection of the uterus occurs in the pathogenesis of the disease. The antimicrobial potential of neutrophils was realease of extracellular defense by activating extracellular defense mechanisms with an active excretion into the extracellular space of the active forms of oxygen and the release of extracellular protective traps. Cytological markers of phagocytic cells (NBT-test, NETs) proved to play or role in pathogenesis of the pyometra should be taken into account during the diagnosis of the pyometra, prognosis of the course of this reproductive pathology and analysis of the adequacy of therapy.

Ethical Approval

This study was approved according to the Law of Ukraine "On the Protection 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 and an informed consent was obtained from the owner prior to the inclusion of the cats in the study. 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).

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

Translated by Mykola Zhelavskyi

Accepted for print 11.05.2020

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