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METHODS FOR REGULATION OF IMMUNE REACTIVITY IN DOGS

MONOGRAPH

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In the modern conditions, immunological blood tests become extremely relevant in clinical practice. By using the results of these tests it is possible to quantify the indicators of non-specific resistance, cellular and humoral immunity, and when studying them in dynamics – to asses the efficiency of treatment courses and develop methods to correct immunodeficiencies. Studies conducted on small domestic animals in large cities have revealed significant changes in immunological status, which leaves an imprint on the course of infectious and non-infectious pathology. The pharmaceutical industry offers a wide range of immunomodulators that affect various parts of innate and adaptive immunity in animals. The key in clinical practice is to determine the individual sensitivity of the animal organism to an immunotropic agent and the prescription of a scheme for immunity correction. It should be noted that violations in the immune system may begin long before the clinical manifestation and manifest themselves under the exposure to a spontaneous biological stimulus. The above indicates the necessity to take into account the formation of adaptive immunity in dogs immediately after birth and, especially in critical periods of the development, emphasizing attention on the period of vaccine administration. The presented results of the author studies allow to gain knowledge about the ambiguity of the mechanisms of regulatory effect of immunoregulators with different origin and plasmapheresis on the functioning of T and B cells in adaptive immunity.

The results of the research presented in this monograph will not only complement the basic knowledge, but will also help veterinarians to better understand the immune system and how it is affected by various agents.

The monograph will be useful to scientists and doctors, as well as to applicants for higher education in the field of veterinary medicine.

Keywords: immunity, immunoglobulins, antibodies, T and B cells, plasmapheresis.

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CONTENTS

LIST OF ABBREVIATIONS

5-HT2B	5-hydroxytryptamine receptor 2B
ATP	adenosine triphosphoric acid
C	control
CAdV	canine adenovirus
cAMP	cyclic adenosine monophosphate
CDV	canine distemper virus
CPV	canine parvovirus
E	experiment
IFN	interferon
IL	interleukin
MHC	major histocompatibility complex
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B cells
NK	natural killer
NKSF	natural killer cell stimulatory factor
NPY	neuropeptide Y
PAMPs	pathogen-associated molecular patterns
p-i	pharmacological interaction of drugs with immune receptors
TLR	Toll-like receptors
TNF	tumor necrosis factor
ІФН	interferon
TCR	T cell receptor

INTRODUCTION

The immune response is a physiological mechanism that protects the whole organism and its individual functional systems and ensures their activity. The ability to protect against foreign agents is caused by the mechanisms of natural resistance or innate immunity, as well as resistance that can develop over time through adaptive immunity. However, when these defense mechanisms do not function properly, this can provoke a disease. For example, hypersensitivity occurs as an excessive and undesirable reaction of the immune system; autoimmunity is activated, which is attributed to violations in mechanisms of immune tolerance that cause the immune system response against own organism.

There are two approaches to solving the problem of adapting the animal organism to environmental conditions: the first is the constant protection of the environment or at least the preservation of its state; the second is to increase the body's resistance to harmful environmental factors. Regarding the second approach, there is considerable interest in substances that stimulate the body's resistance and can mobilize its reserve mechanisms that are inactive under normal conditions.

Currently, chemotherapy is the most common and acceptable approach to control microbial infections of veterinary and medical importance. According to the World Health Organization, antibiotics will soon be ineffective due to the rapid emergence of drug-resistant strains of microorganisms. Antibiotic resistance is an extremely urgent problem of this decade, and in order to survive, it is necessary to seek for alternatives to control pathogens, as well as to protect human and animal health. In this case, immunomodulation with the aim of improving the body's immune potential remains the only alternative to control infection.

Therefore, immunomodulation is of great importance for veterinary science and practice and requires detailed study. This will contribute to the development of compounds that can improve immunity in extremely changing environmental conditions. The ideal immunomodulator has not yet been invented, developed, or validated. Depending on the situation, the immunomodulator can be used to both stimulate and suppress immunity in order to help the macroorganism maintain

homeostasis. Many recognized natural compounds, which are claimed to have an immunostimulatory effect, are currently circulating on the market. However, the question of the effects and application of these compounds can lead many doctors into confusion. Many of these drugs are produced by companies only for financial gain. That is why a specialist in the relevant field must have a thorough knowledge of both positive and side effects and an idea of the methods for their comparison on specific examples. The abovementioned aspects are the focus of research, the results of which are presented in the monograph.

In the authors' opinion, scientific developments on immunomodulators with an emphasis on herbal preparations should be directed and implemented (patented), as they are environmentally friendly and can be an effective means in the practice of veterinary or human medicine. Attention should be paid to a thorough study of the mechanisms of action of powerful and new immunomodulatory molecules, such as cytokines, hormones, protective peptides, Toll-like receptors (TLR), probiotics, nutrients, herbs, polysaccharides, and others that will promote the development of an effective immune prophylactic means, increase vaccine-induced and general immunity, resistance to diseases, and preserving the animal health from various diseases, disorders, and stress.

Dogs are an important experimental biological object for humans. The above is based on the fact that they have similar immune, oncological, infectious, and parasitic diseases, as well as the mechanisms of the disease course. Therefore, the results of the research presented in the monograph can be useful in interpreting the scientific data and drawing parallels between "dogs and humans" in the medical aspect for the benefit of human health.

1. Correction of Immunophysiological Status in Dogs and Prospects for Improving its Efficiency

In modern human and veterinary medicine, immunology occupies a significant place as a developing branch. Doctors of various specialties take it as a baseline. The relevance of the research topic lies in the statement that violations in immunocompetent cell differentiation, their functioning, synthesis of products, or regulation of these processes lead to immunological dysfunctions [1].

Many aspects of the canine immune system remain unknown, namely: the characteristics of the innate immune response, and the role of inflammation in the development of autoimmune and tumor diseases in elderly dogs [2]. In addition, the impact of improved living conditions and regular vaccinations on the activity of the canine immune system is unknown [3]. Knowledge about the functional capabilities of the immune system at different life stages may help veterinarians outline preventive and therapeutic approaches to improve the health, longevity, and quality of dog life [4]. It is extremely important to study the neonatal and geriatric immune system and its ability to respond to a variety of antigens that threaten the lives of newborns and elderly dogs [5]. In addition, dogs are an important animal model for biomedical research, because they have similar immune, tumor, infectious, and parasitic diseases that allow studying many aspects in a short period of time. The similarity between human and canine immunity makes the dog an important research model in developing strategies to improve human life, especially in old age [6].

The most consistent results of recent studies indicate a violation of cell-mediated immune function with age. As a rule, older dogs and cats have a reduced level of $CD4^+$ T cells in blood (with an imbalance in the functional activity of Th1 and Th2), an increase in $CD8^+$ subgroup, and a decrease in CD4/CD8 ratio [7].

Another characteristic feature of modern life in a developed world is access to a high level of nutrition or large amounts of food, as well as its negative aspect – obesity with concomitant effects that contribute to the development of diseases in humans and their pets [8].

The immune system in human and large animal newborns is immature, so the reliability of research results in puppies is more relevant, and these studies can be used for a better understanding immunology of human development [9]. This advantage over mice was recently used to develop extracorporeal support technologies using newborn lambs, the ultimate goal of which was to apply these technologies to premature infants [10].

The most important characteristic of a dog as a medical model is the increase in morbidity with age. The dog is the only species other than humans where individuals exceed evolutionarily determined longevity limits, inevitably creating cancer-prone phenotypes.

In addition, dogs are companion animals and, as a result, are exposed to many of the same environmental conditions and epigenetic influences as their owners. For example, studies have shown consistency between microbiomes in humans and their companion dogs. An interesting fact is that various immune-mediated skin diseases, such as psoriasis and eczema, have also been associated with pet owners [11, 12].

The study of interactions between stress hormones and immune functions is quite relevant. These studies are crucial to increasing knowledge about the mechanisms of disease control in animals, especially in light of the escalation of anthropogenic changes in the biosphere. Indeed, climate change, the introduction of non-native species, the impact of pollutants and changes in habitats, especially urbanization, – all this can change the response to stress and cause disease [13].

The clinical significance of stress in small animals has not been studied, but it is probably that the effect of stress on clinical outcomes such as survival or recovery rate after surgery is underestimated. Consequences of stress for human and animal health serve as a baseline from which concerns for veterinary patients can be extrapolated. Reducing stress in companion animals, which are under veterinary supervision, is undoubtedly important for their mental well-being, and even more important if pharmacological or non-pharmacological measures can prevent disease or improve outcomes of veterinary care [14, 15].

Antibiotic resistance in microorganisms is a modern challenge for scientists and clinicians. In modern conditions, antibiotic resistance (according to the World Health Organization) is one of the most serious threats to human health. Antibiotic resistance is growing every year. The reason for this was the excessive and uncontrolled use of antibiotics in human and veterinary medicine, agriculture, as well as their entry into soil and water [16].

It should be noted that there is no possibility of solving the above-mentioned problem since microorganisms have a significant advantage over antibiotics. They reproduce extremely quickly which contributes to the selection of drug-resistant strains. As a result, antimicrobial drugs become a type of medicinal product whose activity decreases over time. In turn, the decrease in antimicrobial activity of drugs, which is caused by antibiotic resistance, leads to changes in the concept of the most effective drugs [17].

The emergence of new infections, the irrational use of antibiotics in medicine, and their widespread use in agriculture contribute to the emergence of microorganisms resistant to antimicrobial drugs. By 2050, deaths from antibiotic-resistant strains are projected to rise to 10 million people a year, which will exceed cancer deaths [18].

One of the critical periods of development in dogs is the period of 2–3 months of age and this is primarily due to the fact that this period includes measures regarding double or triple immunizations. Also, at this age, there is a switch from fetal to adult hemoglobin in the organism and physiological anemia associated with this process. Blood tests in puppies showed that 65% of blood hemoglobin levels were below the normal range [19].

Vaccination of animals in any case leads to changes in immune parameters in the post-vaccination period and potentially can lead to one of a wide range of possible adverse effects in a small number of newborns [20]. Some studies of the effects of vaccination on the immune system in puppies have shown changes in lymphocyte count. For example, in one study, there was lymphopenia and an increase in lymphocyte reactivity to mitogen in 7 days after vaccination, other studies during vaccination in puppies showed no response in $CD4^+$ lymphocytes and IgG [21, 22].

The dynamics of immunocompetent cells in puppies before and on day 14 after vaccination depended on their number before vaccination [23].

Studies performed in 978 adult dogs, 2–6 years of age, showed that vaccination against canine parvovirus type 2 (CPV-2), canine distemper virus (CDV), and canine adenovirus type 1 (CAdV-1) caused ambiguous antibody formation in the organism. The antibody titer was significantly higher in dogs weighing up to 5 kg compared with the group of dogs, where the average body weight was 10–19.9 kg [24].

According to some reports, a negative effect on immunity formation has adjuvants [25], and according to others – immunosuppression that occurs against the background of vaccine administration and causes diseases, including autoimmune dysfunction. The most pronounced is the effect of the vaccine on T cell subpopulations [26]. Stray animals, the number of which is quite large in Ukraine [27], also significantly affect the immune status in owned dogs, as they have common walking places. In this regard, there is a need to prevent possible complications during vaccination by correcting immunity.

There are many unresolved issues in animal immunity correction, namely: the diagnosis of immunodeficiency disorders and substantiation of the use of drugs with immunotropic activity. Immunity correction can be carried out both independently and combined. Thus, immunity correction is a complex of etiotropic and pathogenetic measures that involve active action on the immunological reactivity of the organism. Immunity correction can be drug, non-drug (nutrition, diet), intracorporeal (within the body), and extracorporeal (outside the body) [28].

In the 1970s, interest in immune-boosting substances increased sharply. This is due to fact that immunostimulants proved to be effective in the treatment of tumors, chronic infections, and autoimmune processes.

Immunomodulators are a group of immunologic agents designed to restore altered functions of the immune system. All immunomodulators, which are used to correct infectious pathology, can be divided into three main groups – exogenous, endogenous, and chemically pure . These compounds are usually combined under the common name “immunomodulators”. The term “immunomodulation” refers to dose-

dependent enhancement or suppression of cellular and humoral immunity, as well as non-specific protective factors [29]. The immunomodulatory effect is reversed and requires the prescription of maintenance doses of drugs [30].

Immunomodulators are used as part of comprehensive treatment in parallel with the prescription of antibiotics, antivirals, antifungals, and other drugs. The strategy of using immunologic agents, based on the modulation of the immune response, has several advantages over traditional antimicrobial treatment, without direct impact on the pathogen. Immunomodulators do not cause the development of polyresistance among microbes, which is very important and relevant today. Due to this, their use can be an important and possible solution to the problem of the rapid spread of antimicrobial resistance [31].

The study and use of immunologic agents revealed the "pendulum phenomenon" – the opposite effect on the immunity of the same means. It turned out that the final result depends on the dose of the drug, time and schedule of administration, initial immune status, genetic characteristics of the organism, as well as the biological species to which the research object belongs (human or animal) [32]. Therefore, today the term "immunomodulators" is more commonly used regarding means that affect immunity. The terms "immunosuppressants" and "immunostimulants" are used only in the case of classification of these substances to indicate their main, predominant effect on the function of immunocompetent cells. Immunomodulators (immunologic agents) include substances of chemical or biological nature that can modulate (stimulate or suppress) immune responses through exposure to immunocompetent cells, their migration processes, or the interaction of such cells or their products (lymphokines and antibodies) with relevant targets [33]. Three variants of immunotherapy are possible – substituted, stimulating, and suppressing. The type of correction is determined by the specific purpose [34]. For example, vaccines or means of substitution therapy (serums and immunoglobulins) are prescribed to prevent infectious diseases; drugs that stimulate immunity are prescribed together with chemotherapeutic agents (especially those that have an immunosuppressive effect – penicillins, streptomycins, tetracyclines, anti-tuberculosis, antifungal antibiotics, co-trimoxazole) in the treatment

of infectious diseases [35]; immunosuppression is applied during transplantation of organs and tissues. Improving the efficiency of basic treatment measures is achieved through combined immunity correction (combination of immunosuppressants with immunostimulants) during autoimmune processes and some oncological diseases [36, 37].

One of the controversial questions remains the mechanism of pharmacological interaction of immunologic agents with immune receptors. Although the exact mechanism is still a matter of debate, the introduction of the drug into the body definitely leads to the activation of drug-specific T cells [32]. Stimulation of B cells or natural killer (NK) cells through binding an immunotropic agent to their immune receptors has not yet been demonstrated [38].

On the other hand, recent scientific data suggests that drug-induced T cell activation leads to reactivation of dormant herpes viruses and that the following symptoms are largely associated with reactivation of herpesvirus infection [34, 39].

Designed to study drug-T cell interactions, drug-specific T cell receptor (TCR) transfectants have demonstrated that T cell activation occurs via TCR [35]. The binding of a medicinal product to the immune receptor occurs according to the same rules as the binding of a medicinal product to a non-immunological receptor. As a rule, drug binding to the receptor occurs rapidly, based on non-covalent interactions, and is reversible [40].

Also raised the question of whether the drug initially binds to the major histocompatibility complex (MHC) molecule (p-i MHC), modifying its structure and thus leading to specific activation of T cells (indirect p-i), or whether the drug binds mainly to specific TCR, which makes the interaction with the MHC necessary but only a complementary signal (p-i TCR, direct p-i) [41].

The importance of the influence of the mediators of the nervous system on the activity of the immune system during the immune response was studied. Immunocompetent cells not only express receptors for neuroendocrine mediators but also secrete many of them. The interaction between the neuroendocrine and immune systems is important for maintaining homeostasis in the organism [42, 43]. Any change

in this interaction can lead to increased susceptibility of the organism to infectious, inflammatory, or autoimmune diseases [44].

There are two main mechanisms by which the central nervous system controls the immune system. The first mechanism includes a hormonal response mediated by the hypothalamic-pituitary-adrenal axis, and the second one – the release of norepinephrine (adrenaline) from the sympathetic nerves and acetylcholine from the parasympathetic nerves, which enter most immune organs (bone marrow, spleen, thymus, and thymus) [45].

The emphasis on the "brain-immune" rather than "immune-brain" communication pathways was conditioned by the active involvement of neuroendocrinologists in this field and the progress made in elucidating the chemical nature and receptor mechanisms of most neuroendocrine factors [46].

Recent studies revealed the immunomodulatory function of the vagus nerve, which activates the efferent arm to regulate cytokine production. This neuroimmune communication is called the "cholinergic anti-inflammatory pathway", provides the host with rapid, discrete, and localized means of controlling the immune response and preventing excessive inflammation [36]. The immunomodulatory ability of the serotonin 5-HT2B receptor on subgroups of human dendritic cells has been shown [37].

Neuropeptide Y (NPY), which is widely distributed in the nervous system, is involved in the regulation of various biological processes, including food intake, energy metabolism, and emotional expression. However, new data suggest that NPY is also a critical transmitter between the nervous system and the immune system, as well as a mediator produced and released by immune cells [28].

Immune system cells express adrenoceptors, which are a target for norepinephrine and adrenaline [47]. Numerous *in vitro* and *in vivo* studies have shown that catecholamines secreted by sympathetic nerve fibers have potent modulatory effects on immune cells. In addition, the latter can produce catecholamines, which act locally as paracrine or autocrine communication factors in similar or different physiological processes [48].

It should also be borne in mind that the functionality of the immune system (as well as other body systems) fluctuates in a circadian rhythm, which must be taken into account when prescribing immunotherapy. Circadian fluctuations in immune system components are an integral regulator that can potentially affect disease onset and therapy [49, 50]. Additional data indicate the importance of daily expression of components of innate immunity for the onset of inflammatory diseases [51, 52].

The thymus and adrenal cortex function in antiphase to each other. The period of best activity of the immune system occurs in the evening [53]. Therefore, in the evening, it is most appropriate to introduce most immunocorrective drugs. However, those immunocorrectives that modulate the synthesis of adrenal hormones (licorice roots, ginseng, Eleutherococcus) should be used in the morning. This corresponds to the physiological period of increased adrenal cortex function [54].

The immune system is characterized by a clear balance of systems and antisystems, a sequence of stages of response. Therefore, when planning the correction of immunologic drugs, it is necessary to clearly determine the clinical and immunological stage of the immune response and the degree of violation of immune mechanisms [55].

The most important achievement of immunopharmacology in the field of immune biotechnology was the production of cell hybrids capable of synthesizing monoclonal antibodies in limited quantities [47]. The use of monoclonal blood pressure in medicine opens up unique prospects for the creation of highly specific and highly effective targeted immunologic agents. There are several classifications of immunologic agents. The difficulties of classification systematics are explained by the fact that these drugs are heterogeneous in chemical structure, mechanisms of action, pharmacological affiliation [7]. In addition, there are many drugs that, along with the main pharmacological effects for which they are used, have immunomodulatory effects. That is why immunologic agents should be divided into suppressive and stimulant [9, 56].

In the field of new immunomodulators there is a slow but steady progress and there is a noticeable shift – the transition from the use of chemically derived drugs to

compounds of natural origin or their analogues: recombinant cytokines, monoclonal antibodies and gene therapy [18].

Immunomodulators of bacterial origin and their semi-synthetic analogues have a longer history of use than synthetic ones, and in recent years their use has become one of the promising areas. This is due to fundamental discoveries in the field of immunology, understanding of the principles of innate immunity based on the recognition of pathogen-associated molecular models of foreign organisms. A wide range of bacteria have been used as immunostimulants, as most of their cellular components act as ligands for various TLRs. As a result, they activate macrophages and dendritic cells, which in turn stimulate the release of a mixture of cytokines [57].

Muramil peptides are bacterial PAMPs because they are fragments of the peptidoglycan (or murein) of all known bacteria. Peptidoglycan is located outside the plasma membrane of bacteria and forms their cell wall. It performs auxiliary and protective functions. Additional residues of fatty acids, peptides and carbohydrates can be found in the peptide chain link of different types of bacteria. Peptidoglycan undergoes structural changes in the process of growth and division of bacteria. These autolytic functions are performed by intracellular peptidoglycan hydrolases and amidases, which destroy peptidoglycan and form muramyl peptides [58, 59].

A significant number of substances of various natures have been studied, including preparations derived from plants, algae, marine aquatic organisms (arabinogalactan, methylan, coral, translam, ponasan, etc.) [60].

A preclinical study of the immunity corrective effect of the amount of active ingredient isolated from essential oils of *Coluria geoides* (Pall.) Ledeb. These drugs had a more pronounced stimulating effect on the synthesis of specific immunoglobulins and proliferation of spleen cells that produce antibodies, compared with the effect of echinacea tincture [61]. Other authors' report the effects of endotoxins from echinacea extracts, which may be an important aspect in the analysis of immunobiological data [62].

The results of immunity correction in dogs with a feed additive that is a combination of krill oil 3%, dried mushrooms (Cordyceps sinensis L.) 2%, krill flour

1%, dried yarrow root (Gentiana Lutea L.) and products obtained by extraction of herbs (Eleutherococcus senticosus) have shown that its use can modulate the immune response Th1 and significantly improve the clinical condition of infected animals [63].

Flavonoids have recently been shown to affect the immune response and may have immunomodulatory effects. They are considered secondary metabolites of plants and have numerous pharmacological functions, including antioxidant, antimutagenic, antibacterial, antiangiogenic, anti-inflammatory, antiallergic, antitumor and enzyme modulation [64, 65].

Among the compounds of natural origin, a group of biologically active substances with humic nature is singled out. It is known that feed additives of humic nature are metabolized and have a multifunctional effect on the body, as they have high adaptogenic properties, maintain immune status, and are actively involved in the regulation of metabolism. The mode of action of humic substances is related to their effect on the cell membrane permeability: they slow down the absorption of organic compounds but enhance the transport of inorganic cations. In the liver, where the primary metabolism of humic substances occurs, the level of cAMP increases, and since it acts as a secondary messenger in the cell, thus humic drugs are able to affect the nuclear apparatus of the cell and thus have a hormone-like effect. The presence of quinoid and polyphenolic groups activates redox processes by increasing the transfer of hydrogen and oxygen in tissues. As a result, the formation of macroergic compounds such as ATP increases, which leads to increased energy processes in the body [66, 67, 68]. Such additives include humic-based biologically active feed additive "Humilid", which was obtained from ecologically pure Ukrainian peat by acid-base extraction [69,70,71].

The main target of immunomodulatory plant products are macrophages, which promote the development of the immune response. Activated macrophages cause enhanced phagocytosis and generate effector molecules such as free radicals, nitric oxide and cytokines that promote intracellular destruction of pathogens. These cytokines may have a direct function or effect on the activity of other populations of immune cells, such as the induction of natural killer cell-mediated cytotoxicity or the

production of cytotoxic T cells. Immunomodulators of plant origin have a huge potential for the creation of new pharmaceutical products [72, 73, 74].

In vivo and in vitro experimental studies have shown that Fosprenil is an effective immunocorrector of secondary immunodeficiencies induced by various external factors: viral infection, stress, radiation [75].

In the period of development and exacerbation of clinical manifestations and dysfunctions of the immune system, it is advisable to use immunomodulatory or immunocorrective therapy. However, if the period of exacerbation is rapid and there is a risk of systemic inflammatory response syndrome, a short course of immunosuppressive therapy (glucocorticoids, infliximab) is appropriate. Method of action and rational use in immune-mediated diseases in dogs are studied for glucocorticoids [76].

It should be noted that glucocorticoids (GC) significantly alter the expression of phenotypic markers on canine lymphocytes and induce apoptosis in vitro. These results determine the potential mechanisms of clinical immunosuppression from glucocorticoid treatment [77]. According to other data, endogenous HA not only suppresses but also directs and enhances immune function. These often overlooked effects may be more important than inhibitory functions in protecting the host and maintaining homeostasis [78]. Systemic glucocorticoid treatment has been associated with susceptibility to infection [79, 80], immunosuppression, and decreased urinary osmolarity [81, 82]. A study evaluating the frequency of side effects and risk factors for systemic HA that occurred during the 31st day of treatment in a large population of dogs during primary veterinary care was conducted in the United Kingdom. These data emphasize that combination systemic glucocorticoid therapy and long-term treatment are associated with an increased risk of adverse events, especially in elderly dogs. Glucocorticoids are commonly used as a first-stage treatment because of their availability, efficiency, and rapid action. However, some patients do not respond to glucocorticoid therapy alone. Others require rapid dose reduction due to serious side effects from HA treatment. These patients benefit from adjuvant therapy [83].

Cyclosporine preparations are licensed for veterinary use [84]. Cyclosporine is a powerful immunosuppressive drug designed to treat autoimmune diseases and organ transplants. In dogs, cyclosporine is used to treat a number of chronic inflammatory and immune-mediated diseases. Cyclosporins are cyclic polypeptide macrolides that were originally derived from the soil fungus Tolypocladium inflatum (Beauveria nivea), but are also produced by other fungi. Cyclosporine A is a molecule designed for commercial use as an immunosuppressive agent [85, 86].

The main immunosuppressive mechanism of action of cyclosporine is the suppression of T-lymphocyte function. Binding of the antigen to CD3 receptors on the surface of T cells causes an increase in intracellular calcium content and activation of calcineurin, an intracellular protein phosphatase that activates gene transcription factors by dephosphorization [87, 88].

By inhibiting calcineurin, cyclosporine specifically inhibits T cell function and thus cell-mediated immunity, but has little direct effect on humoral immunity [89, 90]. Decreased IL-2 expression in CD4 + Th1 cells associated with cyclosporine treatment leads to inhibition of proliferation and activation of both T helper cells and T-cytotoxic lymphocytes [91]. Cytokines are one of the groups of biologically active substances that have been widely tested recently in veterinary medicine. These are hormone-like glycoproteins with low molecular weight, which are necessary for the proper functioning of the immune system [92]. Various immune cells produce them for the interaction and organization of immune attacks [93]. Through unbalanced interaction or abnormal synthesis of cytokines can be observed initiation and continuation of autoimmune and infectious diseases with tumor growth [94].

The role of cytokines as immunomodulators is considered important for future treatment. They have autocrine, paracrine or endocrine effects and can have both synergistic and antagonistic effects [95].

Interestingly, cytokines represent an improved alternative strategy for the treatment of new pathogens. Cytokines can be used to boost immunity and treat or immunocompromised patients, as well as to enhance or induce a desired immune response during vaccination [96]. Modulated cytokine secretion may offer new

approaches to the treatment of a wide range of diseases [97]. The modulation or intervention of the immune response may be significantly affected by the administration of anti-inflammatory cytokines, namely interferon (IFN-13); growth factors (transforming growth factor β), IL-4 and IL-10; or by neutralizing proinflammatory cytokines (IL-2, IFN-γ, IL-12, TNF-α, IL-13 and IL-17) [98].

A set of recombinant cytokines is available for laboratory studies, but specific for the treatment of dogs are absent [99]. IL-12 was originally described as a natural stimulating factor for killer cells because of its ability to promote NK cell activation; this cytokine plays an important role in the induction of IFN-γ, T and NK cell production [100], as well as in the differentiation of Th1 CD4 ± T cells [101].

Studies have shown that IL-12 has potential therapeutic benefits in cancer [102], infectious and inflammatory diseases, and as a vaccine adjuvant [103]. Thus, the production of recombinant IL-12 has aroused great interest in the scientific community.

Nucleic acids are the most important components of the body's immunological homeostasis, as the transfer of genetic information is realized from DNA to RNA. Disorder of nucleic metabolism is one of the causes of induction of pathological processes. Sodium nucleinate (from yeast RNA) enhances phagocytic activity, activates mono- and poly-nucleases, has interferonogenic and antiviral activity, which accelerates the formation of vaccine immunity, reduces the dose of the vaccine.

Sodium nucleinate is released from baker’s yeast, it has the activity of polyclonal immunostimulant, regulating the migration of T cells and the cooperation of T and B cells, enhances the phagocytic activity of macrophages and the production of non-specific protection factors. Has a wide range of biological activity. Accelerates tissue regeneration, stimulates bone marrow and leukopoiesis [104].

One of the potential correctors of impaired protective functions of the body is the drug Derinat, which is a sodium salt of native DNA with a molecular weight of 270–500 kDa. Derinat is obtained from the milk of salmon or sturgeon. It is a unique polymeric immunomodulator that also has radioprotective, antiviral, regenerative activity. According to current data, Derinat, introduced into the body, enters cells by pinocytosis, followed by processing and cleavage to nucleotides, which after release

into the extracellular environment bind to purinergic P2 receptors, represented by families of P2X and P2Y receptors and those expressed almost on all cells of the body [105, 106].

Currently, nucleotides, in addition to the classical characteristics as carriers of genetic information and participants in energy metabolism, are considered as a type of signaling molecules that play an important role in the regulation of immunity [107]. This determines the broad prospects for the therapeutic use of the drug Derinat for the correction of impaired immune function and, therefore, the need for in-depth study of its mechanisms of action. The greatest interest is the study of its effects are not normal, but by changes in the degree of activity of the immune system.

Various chemicals with immunomodulatory properties have been reported in the literature . For example, levamisole can have an effect on both the patient and the normal immune system, so it is both an immunostimulant and an immunoregulator. Its effect on T cells is relatively more significant than on B cells. Levamisole has been used to stimulate immune cells in various cancers [108].

Recently synthesized a new veterinary drug Trifuzol from the group of 1,2,4-triazole derivatives showed promising results in terms of high immunomodulatory activity, antioxidant properties, hepatoprotective properties with pronounced pancreatoprotective effect and, in particular, significant antiviral effect on some viruses. The effect of 1,2,4-triazole on reducing the titer of specific antibodies against CDV and CPV in the blood of puppies can be considered as a consequence of antiviral activity [109].

It is well known that nutrition plays a significant role in immunomodulation, and malnutrition is the most common cause of immunodeficiency worldwide. Therefore, the proper functioning of the immune system requires a sufficient supply of nutrients and both deficiency and excess of nutrients adversely affect the various components of the immune system. This is due to the fact that the optimal functioning of the immune system includes a variety of biological actions that stimulate cell growth, energy metabolism, production of proteins and antioxidants. Arginine is important for the role of amino acids in the oxygen killing of microbes in phagocytic cells [110-112].

Glutamine is necessary for the proper functioning of lymphocytes and macrophages and the induction of the immune system during inflammation. The use of glutamine by macrophages and lymphocytes during inflammation has also been reported to be high. Glutamine is also required for the secretion of cytokines and antibodies, as well as for cell division [113].

Data on the effect of dipeptide with anxiolytic activity (GB-115, N-phenylhexanoyl-glycyl-L-tryptophan amide) on immune parameters in intact mice and animals with secondary immunodeficiency caused by cyclophosphamide at doses of 0.1–10.0 mg/kg indicate that it stimulates the phagocytic activity of peritoneal macrophages and the humoral immune response in intact mice. GB-115 also showed immunocorrective activity in animals with secondary immunodeficiency [114]. Essential fatty acids in food can strengthen the immune system and maintain health [115].

Linoleic acid is a precursor to omega-6 and is present in vegetable oils and soybeans. In the plasma membrane of immune cells, linoleic acid is converted to arachidonic acid. Therefore, omega-6 polyunsaturated fatty acids have an inflammatory effect that is the opposite of omega-3. These results indicate that a diet high in omega-3 PUFAs reduces inflammation by increasing docosahexaenoic and eicosapentaenoic acid levels in the plasma membrane by inhibiting arachidonic acid [116, 117], and omega-3 PUFAs reduce inflammatory and autoimmune disorders. [118]. Fatty acids and amino acids, together with other secondary plant substances, namely carotenoids, flavonoids and spices, inhibit the release of pre-stored mast cell mediators such as histamine or de novo expression of mast cell mediators such as cytokines and eicosanoids. for the prevention of allergic diseases [119].

Trace elements important for immune function include vitamins A, C, E and B6, copper, folic acid, iron, selenium and zinc. Other nutrients, such as beta-carotene (precursor of vitamin A), vitamins B12 and D, also play an important role in immune function. They typically promote the body's natural defenses on three levels, affecting physical barriers (skin / mucous membranes) and innate immune cells, cell-mediated immunity, and humoral immunity. Substances such as vitamins A, C, E and the trace

element zinc are needed to strengthen the skin's barrier function. Vitamins A, C, D, E, B6, B12, folic acid and trace elements, in particular copper, iron, selenium and zinc, act synergistically to support the protective activity of immune cells. All of these micronutrients (except iron and vitamin C) are needed to make antibodies. Copper, selenium, zinc and vitamin B6 play important roles in B cell proliferation and antibody production. Vitamins A, D and E enhance the Th2 response, which leads to enhanced humoral immunity. Other microelements are indirectly involved in protein synthesis and cell growth [120, 121].

Scientific evidence suggests that short-chain fatty acids are able to enhance the synthesis of protective peptides in porcine intestinal epithelial cells and alveolar macrophages, suggesting the importance of fatty acids as immunostimulants in both humans and animals [122].

Vitamin A plays an important role in maintaining the integrity of the surfaces of the mucous membranes of the respiratory tract and gastrointestinal tract, as well as in the regulation of innate and adaptive immune response [123]. Vitamin A and its metabolites such as transretinoic acid and retinol and retinoic acid play an important role in both cellular and humoral immunity. They also enhance phagocytic activity and regulate immune homeostasis through peripheral induction of regulatory T cells. In addition, they can control the differentiation of CD4 T cells; mucosal immunity and immune tolerance, and induce regulatory and inflammatory responses [124].

Vitamin A deficiency leads to a decrease in phagocytic and oxidative activity of activated macrophages during inflammation [125] and a decrease in the number of natural killer cells and their activity [126]. Vitamin A deficiency severely weakens humoral immunity [127] and provokes inflammation in the body due to increased secretion of TNF-α and IL-12. But vitamin A can be a reducer of these processes [128].

Most immune system cells, such as T cells and macrophages (except B cells), express vitamin D receptors in significant concentrations [129, 130]. Vitamin D has an immunomodulatory effect, inhibiting excessive production of inflammatory cytokines and enhancing the oxidative activity of macrophages. In addition, it also induces the secretion of potent antimicrobial peptides in most immunocompetent cells [131].

Vitamin E has strong lipid-soluble antioxidant activity that eliminates free radicals and can enhance both cell division and cytokine secretion by naive T cells but not memory T cells. Vitamin E can also inhibit a variety of inflammatory processes by blocking the activity of the NF-kB transcription factor, which is important for the transcription of many proteins, especially proinflammatory cytokines. Vitamin E promotes an immune response that targets Thl immunity [132].

Leukocytes are enriched with vitamin C, which is the main water-soluble antioxidant in cells and plasma. It performs its antioxidant action by purifying reactive oxygen species formed during the process of phagocytosis by activated immune cells [133]. The addition of vitamin C enhances the various components of the immune response, and therefore, in addition to its metabolic functions, its significant role in immune homeostasis cannot be neglected [134].

A powerful cytokine-like immune regulator is leptin, which has complex effects such as an inflammatory response to both malnutrition and overeating [135].

One of the immunologic agents is interferon, which in the form of a pharmacological agent is used to affect the immune system in various forms of dysfunction of the body. Studies using IFN subtypes have identified different properties of the protein with different antiviral efficacy, opening promising pathways for immunotherapy [136]. Recombinant canine IFN-γ is considered an effective treatment for atopic dermatitis in dogs [137].

Type I interferons, such as IFN-α, IFN-β, IFN-ε, IFN-κ and IFN-ω, are cytokines involved in the regulation and activation of innate and adaptive immune responses. They have strong antiviral, antiproliferative and immunomodulatory activity, which allows their use in the treatment of viral diseases, tumors and immune-mediated diseases. Previously, treatment strategies were based on non-specific inducers of IFN type I, later they were replaced by various recombinant proteins [136, 138]. However, strong activation of the IFN-I signaling cascade, especially in activated microglia / macrophages, is likely to contribute to immune-mediated demyelination mechanisms in the later stages of the disease. Studies have shown that many viruses, which were

generally thought to cause only acute transient clinical infections, sometimes persist in sequestered areas in residual amounts without any apparent effect [139].

In vivo experiments have demonstrated the therapeutic efficacy of IFN-α in the treatment of viral infections such as respiratory infections in cattle, transmissible gastroenteritis or rotavirus diarrhea in pigs, other viral infections in mice, and immunocompromised diseases such as polymyositis and experimental autoimmune allergic encephalomyelitis in rats and mice [140, 141].

The use of low doses of oral IFN-α has also been shown to be useful in the treatment of cats with feline leukemia virus and feline immunodeficiency virus, which has led to dramatic improvements in the health of subjects [142].

In dogs with dry keratoconjunctivitis, which are mainly treated with surgical correction or artificial tears, oral administration of IFN-α has been shown to increase tearing, making it an effective therapeutic alternative for the treatment of this disease [143].

Another study demonstrated the efficiency of oral IFN-α treatment in dogs with idiopathic recurrent superficial pyoderma in terms of improving clinical averages and reducing the need for antimicrobials [144].

Studies have been performed to evaluate the therapeutic efficacy of low-dose oral recombinant canine IFN-α subtype 4 (CaIFN-α4) in dogs with periodontal disease. The mechanism of its effect on the immune system is expressed in the ability to enhance the production of IL-8 by gum fibroblasts in response to bacterial and cytokine components that modulate inflammatory processes in periodontal tissues. There are data on favorable results in the treatment of parvovirus infection in dogs [145] using 2.5 IU / kg of recombinant canine IFN-α for three days.

One of the active ingredients responsible for the immunomodulatory effects of many herbs are complex polysaccharides known as "β-D-glucan" or simply β-glucan Receptors and mechanisms of action of β-glucans have been revealed in in vitro and in vivo experiments on animals. β-glucans are ubiquitous in both bacterial and fungal cell walls and are involved in the initiation of the antimicrobial immune response. β-glucan of high purity (PLEURAN, Bratislava) had a positive effect on immunological

parameters; β- (1,3 / 1,6) -D-glucan administered at a dose of 2 mL / 5 kg body weight caused, in addition to a marked improvement in phagocytic function, a significant increase in CPV titers and antirabies antibodies, which remained until the end of the experiment [146].

In the 1980s, Japanese virologists discovered the alkaloid granicidin in the peel of the world's largest citrus, called Citrus grandis. Based on the obtained data, the immunomodulatory drug Cycloferon was developed. Its administration (12.5%) at a dose of 10 mg / kg body weight (0.7 mL per animal) subcutaneously according to the scheme provided by the instructions, namely, 1-, 2-, 4-, 6-, 8- and On the 10th day, it causes significant T helper cell activity and reduces the ability of neutrophils to phagocytize [147].

In the period of convalescence, when the antigenic load is significantly reduced, it is possible to conduct immunostimulatory therapy. In addition, it is during this period that it is most appropriate to use immunologic agents with a vaccine effect or those obtained on the basis of animal and plant extracts [148].

An important factor in the use of immunologic agents is their focus on the elimination of antigen. Elimination of the immune response does not always lead to the return of the body to normal. For example, after release from the virus, the body experiences a temporary state of immunosuppression associated with lymphopenia, with the immune system switching to the T-helper 2 response, making the body susceptible to other infections [149]. Thus, infections and immune responses to them may have consequences that persist after the release of the pathogen.

One of the modern pharmacological means of immunomodulatory effect are drugs based on lactoferrin - a glycoprotein of colostrum, which enhances phagocytic and cytolytic functions aimed at eliminating the pathogen, has a bacteriostatic effect, activates the synthesis of IL-1, accelerates cell maturation. to enhance cellular immune defense against viruses, endotoxins and exotoxins), shifts the immune response from Th2 to Th1 phase, stimulates the production of interferon by disrupting the replication of viruses. In the presence of exogenous LF, the expression of a number of cytokines changes; its interaction with some receptors causes the activation of immune cells and

the synthesis of molecules on the surface of endothelial cells that mobilize and direct leukocytes to foci of inflammation [150].

The reason for the variety of chronic diseases is a violation of homeostasis due to either excessive intake of xenobiotics, including toxic, from the outside, or violation of various defenses - detoxification, immunity, excretion of pathological products from the body [151]. Without eliminating the causes of immunosuppression or distorted immune responses, it is difficult to count on sustained immunity correction. If you do not rehabilitate the internal environment, do not remove pathological products, do not restore normal metabolism, including lipid peroxidation or proteolysis, if you do not eliminate the "toxic pressure" on the immune system is difficult to recover only with drug stimulation [152].

On the one hand, detoxification therapy helps to eliminate metabolic immunosuppression, and on the other - increases the efficiency of the immunostimulatory effect of drugs. If immunostimulating drugs are used against the background of severe intoxication, a paradoxical deepening of the immune defect can be obtained due to the triggering of pathological apoptosis (programmed cell death) of immunocompetent cells, which once again confirms the important role of the previous stage of detoxification. It is possible to use both parenteral (colloids, crystalloids, diuretics) and oral detoxification (enterosgel, polysorb, silica, dufalak-vet). Some immunologic agents combine immunomodulatory effect with detoxifying action (polyoxidonium, dufalak) [153].

However, not all substances to be removed from the body can be captured and fixed on sorbents. Electrochemically inert molecules are not capable of adhesion and remain in the circulation, which makes the hemosorption procedure incomplete. In these cases, the effect of elimination of such substances can be obtained by plasmapheresis, when a certain part of the blood plasma is completely removed together with all the pathological products contained in it. The removed plasma volume is filled with plasma replacement solutions, albumin or donor plasma. In the latter case, especially when the removed plasma is completely replaced by donor, the operation is called plasma exchange. Membrane plasmapheresis has been used in recent decades in

the clinic of small animals [154]. There are almost no data in the literature on the effect of such a procedure on changes in physiological parameters of cellular immunity. But in contrast to hemosorption, plasmapheresis is more universal, when all pathological products are removed, regardless of the presence and magnitude of the electrostatic charge of their molecules [155].

Plasmapheresis is an auxiliary method of intensive correction of physiological functions of the body. This method of blood purification is suitable for both animals with surgical pathologies and therapeutic patients. Positive dynamics is observed during several plasmapheresis procedures.

For therapeutic purposes, plasmapheresis is performed in many diseases and pathological conditions (for example, in exogenous intoxications - food poisoning, drug overdose, after chemotherapy; in endogenous intoxications - severe diseases accompanied by severe intoxication, namely: osteomyelitis, osteomyelitis) [156, 157, 158, 159]. Double filtration plasmapheresis was performed in dogs with leishmaniasis, resulting in rapid elimination of signs of hyperproteinemia [160]. Canine leishmaniasis is a disease caused by Leishmania infantum, which is often associated with glomerulonephritis due to the deposition of immune complexes in the nephrons [161].

For complete remediation of the internal environment usually requires 4 sessions of plasmapheresis with an interval between procedures of 1-2 days. In this mode, even when replacing plasma only with isotonic sodium chloride solution, there are no significant changes in the main components of the internal environment (proteins, fats, carbohydrates, electrolytes, hormones, etc.). Newly formed cellular and humoral elements of homeostasis in an "updated" environment, devoid of "toxic pressure" of removed pathological products, retain their long-term natural functions and properties.

However, for all its importance, efferent therapy aimed at removing pathological products of the internal environment is only the first step in correcting disorders. The second - the elimination of the secondary effects of these violations - the restoration of natural defense systems, mainly immunity.

According to research, another effective method of immunity correction is the use of liposomal emulsions. In 1971, the first attempts to include enzymes in liposomes

were made. Later, liposomal forms of some antitumor drugs, antibiotics, hormones were developed. Today the direction of practical use of liposomes is developing very actively. The ability of liposomes to contain a variety of substances with almost no restrictions on their chemical nature, properties and size of molecules provides unique opportunities to solve some problems associated with the frequency of injections of immunologic agents [162].

The inclusion of drugs in liposomes can significantly increase the therapeutic efficacy of the former, because, on the one hand, the drug contained in the liposome is protected by its membrane from adverse factors, and on the other - the same membrane does not allow toxic drugs to exceed acceptable concentrations in body fluids organism [163]. The liposome acts as a carrier from which the drug is released gradually, in the right doses and places, over a period of time.

Liposomes are closed spherical vesicles formed from amphiphilic substances, such as phospholipids (diphylphilia or amphiphilicity - the ability of some parts of the macromolecule to prefer, for example, the polar environment and others - non-polar). When interacting with aqueous solutions, these substances self-organize into two-layer membranes [164]. Liposomes effectively protect the substances included in them from contact with the body's enzyme systems, preventing premature inactivation of the drug [165]. Thus, liposomes give rise to a new way of targeting the cell, which can be called "membrane engineering".

A separate direction of drug administration is transdermal administration of liposomes, which, in contrast to intravenous, causes a stronger immune response [109].

As carriers of drugs liposomes have a number of advantages: they protect body cells from the toxic effects of drugs, prolong the action of drugs administered into the body, protect drugs from degradation, promote targeted specificity due to selective permeability from blood to tissues, change pharmacokinetics of drugs. increasing their pharmacological efficiency, allow to create a water-soluble form of a number of medicinal substances, thus increasing their bioavailability .A new promising area is the development of immunoliposomes [166]. These are liposomes to which monoclonal antibodies are attached . The latter provide specific binding of liposomes to antigen-

positive cells, and liposomes deliver the appropriate hydrophobic or hydrophilic chemotherapeutic drug [167].

The "ideal" liposome model as a means of targeted drug delivery to a cell includes an internal drug, flexible polymer chains immobilized on its surface to reduce uptake by RES cells, and fusion proteins incorporated into the membrane to recognize certain cells. .

In the scientific literature, there are not enough discussions regarding the researches of estrus as a physiological stressor for the bitches, because unlike other animals (cows, sheep, pigs), it runs for a longer period. Data from laboratory, clinical, and epidemiological studies of this process suggests that strong and chronic psychogenic stress affects animal and human health, including susceptibility to infections [168] and delayed wound healing [169]. The importance of understanding the stressor of estrus is also related to the frequent development of inflammatory processes in the uterus after the estrus and the premature impossibility of reproduction in bitches. In some countries, this disease develops in almost 25% of bitches under the age of 10 [170].

The normal course of the sexual cycle is also affected by the functional state of the thyroid gland. Thyroid hormones also have a documented effect on the secretion of hormones involved in the reproduction and maintenance of pregnancy. The action of thyroid hormones is explained by the presence of thyroid-stimulating hormone and thyroxine receptors in human ovarian tissue [171]. Thyroid hormone receptors were identified in oocytes, and their deficiency may affect fertilization performance.

There are many factors that lead to violations of the sexual cycle in bitches. Such factors also include adequate interaction between the links of the local (mucous membrane of the reproductive organs) and general immunity in the body of the bitches and between the immune and endocrine systems [172].

Therefore, since many issues of immunity correction remain unexplored, there is a need for further study and solution of problems in the development of optimal schemes and tactics for immunity correction. It is also important to develop indicative

methods of immunity correction, taking into account the individual sensitivity of the organism to biological substances with immunotropic action.

2. Assessment of the Degree of Sensitization in Dogs to Biologically Active Substances with Immunotropic Action

In our author experiments, studies were performed using loading tests, where biologically active substances with immunotropic action of various origins were used as antigens. The aim of this experiment was to establish the level of autosensitization of dogs to antigens, which will predict the development of the immune response to the introduction of biological substances into the body.

Biologically active substances most often offered by veterinary pharmaceutical production were used as antigens. It is established that the degree of autosensitization to the studied immunologic agents in the body of dogs has its own characteristics (Table 1).

It should be noted that 13% of animals (8 dogs) had very low sensitivity to Roncoleukin – within 5%, in contrast to Imunofan and Fosprenil, to which 6% of experimental animals had very low sensitivity (4 dogs out of 50 and 64 dogs, respectively). Analysis of the degree of autosensitization to immunologic agents in the range of 5–10 and 10–16% showed that 23.5% of animals had such sensitivity to each degree to Fosprenil, 16 and 34% – to Imunofan, 14 and 18 % – Polyferon, and 20 and 30% – Roncoleukin. On average, this was 18.4 ± 4.23 and 28.9 ± 4.36% ($p < 0.01$), respectively.

Table 1

Indicators of the degree of sensitization of the body to immunomodulators

Drugs	Degree of individual sensitivity, %									
	< 5		5–10		10–16		17–40		40 <	
	No	%	No	%	No	%	No	%	No	%

Roncoleukin	8	13	13	20	19	30	22	34	2	3
Polyferon	5	8	9	14	18	28	28	44	4	6
Imunofan	4	6	10	16	22	34	26	41	2	3
Fosprenil	4	6	15	23,5	15	23,5	27	42	3***	5
M ± m, %	8.3 ± 2.83		18.4 ± 4.23*		28.9 ± 4.36**		40.3 ± 4.34		4.3 ± 0.83	

Note: 1. *p < 0.01 significant difference between sensitivity < 5, 5–10, and 17–40%; **p < 0.001 significant difference between sensitivity 5–10 and 10–16%.

2. ***the administration of this drug to animals caused clinical manifestations of hypersensitivity reaction.

3. No – number of animals.

The results of the analysis of autosensitization to biologically active substances in the range of 17–40% show that this degree had the largest number of animals – 40.3 ± 4.34% (p < 0.05). The percentage of such animals is higher in the case of the drug Polyferon, although the difference between other drugs was insignificant and amounted to 2–3%, except for the drug Roncoleukin (8%). However, to the last drug the smallest number of animals (2 dogs) had a sensitivity above 40%, while to the immunocorrector Polyferon – 4 dogs.

The difference between the averages for each drug did not differ significantly from each individual and was, on average, ± 5%. This may indicate that for most immunocorrective drugs, individual immunoreactivity will be within these limits. It should be noted that animals that during the study had a degree of sensitization to drugs more than 40%, their introduction is risky because it can lead to a hypersensitivity reaction.

We analyzed the immunograms of dogs of different ages in order to establish the features of T-reception of lymphocytes to biologically active substances with immunotropic action. Analysis of data on autosensitization of dogs to biologically active substances in dogs at different ages showed that at the age of one year the number of animals had low (up to 10%), medium (10–16%) and high (more than 17%) the degree of autosensitization to immunologic agents was 29, 38, 33%, according to each variant of autosensitization. A study of this indicator in mature animals (1–6

years) showed an increase of, on average, 5% in the number of animals that had a medium (10–16%) and high (more than 17%) degree of self-sensitization compared with younger animals. After six years, when the process of thymic involution naturally accelerates in dogs, it was found that the number of dogs with high (more than 17%) individual sensitivity to immunologic agents increased to 58% against 33% at a young age. This is an important fact that must be taken into account when prescribing immunologic agents during immunity correction in order to prevent the possible manifestation of adverse effects.

Thus, the obtained data indicate the presence of age features and a certain pattern in the sensitivity of the organism to immunologic agents, which is expressed in the T-reception of activated lymphocytes.

3. Influence of the Immunologic Agent on the Activity of T Cell Receptors

Studies conducted by the authors have shown that there is an individual sensitivity in dogs to different immunologic agents. On average, about 25% of dogs had low individual sensitivity to different immunity correction, 70% – high sensitivity, and 5% – excessive sensitivity (the administration of a drug to which they have excessive individual sensitivity usually causes anaphylactic shock) [173].

There are no data in the available literature on the effect of the administration of immunologic agents in recommended doses during their use on the change in individual sensitivity of the organism, namely the activity of T cell receptors. First of all, the main indicators of immunograms in experimental and control animals were determined, as well as the degree of individual sensitivity (according to loading tests) to immunologic agents.

When determining the main physiological parameters of cellular immunity (Table 2), it was found that the absolute number of leukocytes in animals of the control group was 9.9 ± 2.78 g/L compared with 8.4 ± 2.75 g/L in animals of the experimental group ($p < 0.01$). The absolute number of lymphocytes in animals of the experimental group was higher by 0.15 g/L ($p < 0.001$) than in the control before drug administration.

Table 2

Dynamics of the absolute number of immunocompetent cells and the activity of T cell receptors after the administration of the immunologic agent (M ± m)

Parameters	Before administration		After administration	
	Control group	Experimental group	Control group	Experimental group
Leukocytes	9.90 ± 2.78	8.40 ± 2.75*	9.33 ± 2.80	10.93 ± 2.05*
Lymphocytes	2.39 ± 0.10	2.64 ± 0.57**	2.53 ± 0.72	3.13 ± 0.23*
T cells	1.74 ± 0.46	2.08 ± 0.61*	1.81 ± 0.37	2.09 ± 0.19
T helper cells	1.36 ± 0.33	1.61 ± 0.32	1.37 ± 0.29	1.57 ± 0.24**
T suppressor cells	0.38 ± 0.04	0.46 ± 0.09	0.44 ± 0.06	0.52 ± 0.05*
B cells	0.30 ± 0.08	0.23 ± 0.05*	0.31 ± 0.09*	0.46 ± 0.05*
NK cells	0.85 ± 0.05	0.14 ± 0.02**	0.12 ± 0.03	0.21 ± 0.06
Phagocytic activity of neutrophils	3.59 ± 0.42	2.37 ± 0.64*	3.77 ± 0.12	3.94 ± 0.54*
Immunoregulatory index (Th/Ts)	3.43 ± 0.06	3.23 ± 0.08	3.67 ± 1.09	3.07 ± 0.060
Sensitivity to drugs, % inversion	12.00 ± 1.15	15.00 ± 4.16	11.00 ± 1.15	8.70 ± 0.50**

Note: *p < 0.01; ** p < 0.001 compared with animals of the control group.

Evaluation of lymphocyte subpopulations before the administration of biologically active substance with immunotropic action showed that the absolute number of T cells in animals of the experimental group increased by 20.11% and was 2.09 ± 0.61 g/L against 1.74 ± 0.46 g/L in the control ($p < 0.01$). However, the absolute number of B cells prevailed by 23.3% in animals of the control group ($p < 0.01$). In animals of the experimental group, the absolute number of NK cells (broad plasma lymphocytes) was significantly reduced – 0.14 ± 0.02 g/L, which is 6 times less than in the control ($p < 0.001$).

In animals of the control group, the phagocytic activity of neutrophils before the administration of the drug was found to be 1.22 g/L higher ($p < 0.001$). After determining individual sensitivity to immunologic agents (according to the percentage

of inversion of activated T cells), it was established that it was optimal (inversion of more than 10%) to the drug Fosprenil.

The next stage of the author studies was the comparison of the immunoreactivity of the animals of the experimental and control groups after a 5-day course of Fosprenil, as well as the establishment of changes in the individual sensitivity of the organism to this drug (Table 2).

An increase in the absolute number of leukocytes in animals of the experimental group by 30.11% ($p < 0.01$) was established after the introduction of the drug. This indicator in animals of the control group had a tendency to decrease by 5.75%.

A more pronounced increase in the absolute number of lymphocytes was noted in animals of the experimental group. Thus, in the control, the absolute number of lymphocytes increased by only 5.86% ($p < 0.01$), and in the experiment – by 18.56% ($p < 0.01$) or 0.49 g/L.

At the same time, changes in the indicators of lymphocyte subpopulations had their own characteristics. In particular, in the dogs of the experimental group, after the course of the immunologic agent, the absolute number of T-helper cells decreased by 2.54% ($p < 0.001$), and T suppressor cells, on the contrary, increased by 12.53% ($p < 0.01$), however in the control group, with an increase in T-helper cells, there was also an increase in the number of T suppressor cells. The absolute number of phagocytic cells increased more markedly in animals of the experimental group.

A significant increase in the absolute number of phagocytic cells was established. In the experimental group, after administration of the drug, it probably increased by 66.31% ($p < 0.01$). In control animals, this indicator had a slight increase of 4.95%.

The dynamics of the absolute number of NK cells after the introduction of "Fosprenil" was somewhat peculiar. Thus, in the control group, their number decreased by 6.07 times, and in the experimental group, at the initial level of 0.14 ± 0.02, there was an insignificant increase to 0.21 ± 0.06.

When studying the changes in the sensitivity of T cells before and after the administration of the immunologic agent, a significant decrease in the activity of the

surface receptors of these cells was noted, which was expressed by a decrease in the percentage of inversion. So, in the control, this indicator decreased by 1.0; and in experimental animals – by 6.3% ($p < 0.001$). The number of B cells doubled ($p < 0.01$) in animals of the experimental group, while the absolute number of these cells remained almost unchanged in the control dogs.

Analysis of the state of the immunoreactivity of dogs before and after the introduction of a biologically active immunologic agent makes it possible to detect immunological changes in the dynamics during immunity correction depending on the degree of autosensitization (by the percentage of inversion of activated T cells). In the table 3 presents the dynamics of the ratio of protein fractions in the blood serum of dogs after the administration of a biologically active drug with an immunotropic effect. As can be seen, in animals of the experimental group, the relative content of albumins decreased by 2.47 ($p < 0.01$), α1-globulins by 1.8% after the administration of the drug. In the control group, on the contrary, there was a tendency to increase the content of albumins by 1.3% and to decrease the content of α1-globulins by 0.36%.

The assessment of the relative content of protein fractions before and after administration of the drug in the experimental and control groups showed that the most pronounced decrease in the content of the γ-globulin fraction, which also refers to metabolites that provide a humoral immune response, was the most pronounced in all animals. At the same time, in the experimental group, the decrease was 1.6 times ($p < 0.01$), while in the control group – 2.3 ($p < 0.001$).

Table 3

The dynamics of the ratio of serum protein fractions in dogs after the administration of the immunologic agent, % (M ± m)

Protein fractions	Before administration		After administration	
	Control group	Experimental group	Control group	Experimental group
Albumins	51.34 ± 10.72	57.34 ± 7.11	52.7 ± 12.93	54.87 ± 9.37*
α_1 globulins	4.98 ± 1.07	4.13 ± 2.18	4.63 ± 0.98	5.93 ± 2.17
α_2 globulins	7.71 ± 4.12	8.39 ± 2.04	8.19 ± 2.86	7.94 ± 2.93

β-globulins	5.82 ± 1.02	7.11 ± 1.19**	5.73 ± 1.84	4.98 ± 1.14
γ-globulins	27.34 ± 3.35	19.81 ± 1.47**	11.93 ± 2.12	12.71 ± 2.18*

Note: *p < 0.01; ** p < 0.001 compared with animals of the control group.

A more moderate decrease in the content of γ-globulins was noted in animals of the experimental group. This is probably due to a more pronounced increase in the absolute number of B cells. This process can be considered as a compensatory reaction based on the principle of positive and negative feedback. According to this principle, the number of cells that synthesize immunoglobulins increases in response to a decrease in the content of immunoglobulins, and vice versa, an increase in the content of immunoglobulins in the blood serum leads to a decrease in the number of B cells.

Thus, during the use of immunotropic agents in the recommended doses, the sensitivity of lymphocytes to them decreases. It is considered an established fact that the effect of most immunotropic agents on the body is receptor-mediated, and only in the presence of tropic receptors is it possible to achieve an immunocorrective effect [174, 175]. The described dynamics of changes should be taken into account when conducting courses of immunity correction for various clinical conditions in small animal veterinary practice.

4. Enhancing the Natural Resistance and Immunological Reactivity in Puppies by Using Humic-Based Feed Additives

Analyzing the data shown in Table 4, it should be noted that the most pronounced changes after applying Humilid, a humic-based biologically active feed additive, related to the content of total serum protein and its γ-globulin fraction.

Table 4

Dynamics of the content of total serum protein and its fractions after application of Humilid, a humic-based biologically active substance (M ± m; n = 3)

Parameters	Before		Day 7		Day 14		Day 21	
	E	C	E	C	E	C	E	C
Total protein, g/L	45.20 ± 2.25	41.6 ± 5.76	45.37 ± 5.57	38.23 ± 6.70	46.77 ± 2.88	33.60 ± 6.41	48.40 ± 6.41	28.80 ± 5.4*
Albumins, %	50.8 ± 3.81	51.7 ± 6.49	48.13 ± 4.62	50.33 ± 5.31	47.43 ± 1.16	48.90 ± 2.08	49.20 ± 3.81	46.0 ± 0.09*
α_1-globulin, %	3.0 ± 0.35	1.30 ± 0.31	2.17 ± 0.23	1.37 ± 0.02	2.40 ± 0.69	0.83 ± 0.27	1.43 ± 0.29*	0.63 ± 0.06*
α_2-globulin, %	2.5 ± 0.69	2.0 ± 0.37	2.97 ± 0.85	2.93 ± 0.75	2.70 ± 0.21	5.57 ± 1.33	2.37 ± 0.12**	0.13 ± 0.02**
β-globulin, %	6.8 ± 1.91	6.23 ± 0.75	5.66 ± 1.37	3.80 ± 0.87	4.77 ± 0.29	3.23 ± 1.44	4.57 ± 0.46*	3.00 ± 0.12*
γ-globulin, %	9.9 ± 1.45	11.3 ± 2.41	12.87 ± 1.85	10.63 ± 2.19	14.73 ± 1.27	14.83 ± 0.40	15.53 ± 1.44*	13.90 ± 0.18*

Note: 1. *$p \leq 0.05$; **$p \leq 0.01$ in compare with indicators before feed additive administration.

2. E – experimental group; C – control group.

Thus, in animals of the experimental group, the content γ-globulins was on average, 9.9 ± 1.45% from total protein before applying this additive, and this figure increased to 15.53 ± 1.44% (difference – 5.63%) after 3 weeks. In the control group, the content of γ-globulins was 11.3 ± 2.41% at the beginning of the experiment and 13.90 ± 0.18% (difference – 2.63%) after 3 weeks. That is, the application of humic-based biologically active substance increases the level of serum γ-globulins in animals of the experimental group by an average of 3% compared with controls. At the same time, similar dynamics was recorded regarding the level of albumin, α_1- and α_2-globulins, as well as β-globulins.

Fig. 1 shows changes in the total number of leukocytes in animals of the experimental and control groups in the course of the experiment. In the animals of the experimental group, there was a more pronounced increase in the population of these blood cells on the 7th and 14th day, in contrast to the control group, in comparison with the value of this indicator at the beginning of the experiment.

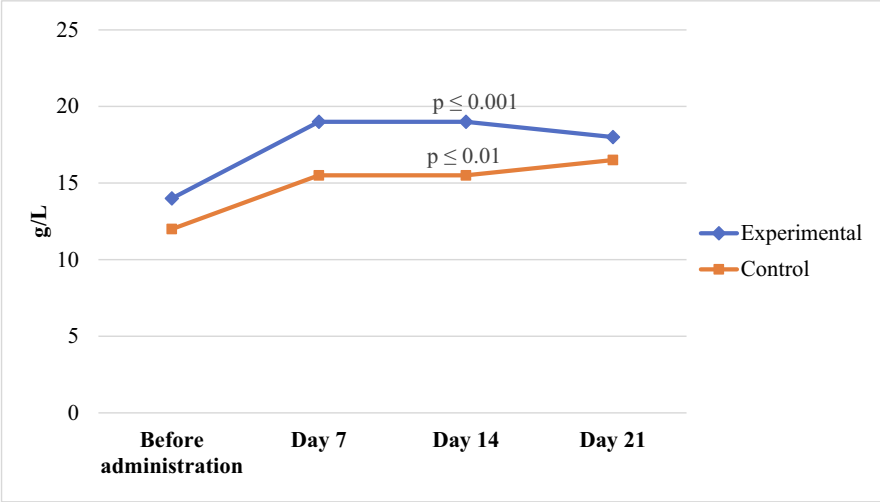


Fig. 1. Dynamics of leukocyte count during the experiment, g/L

The same dynamics were observed during the analysis of the absolute number of granulocytes in animals of the experimental and control groups (Fig. 2).

The monocyte population during application of the humic-based biologically active substance Humilid (Fig. 3) increases throughout the experiment in both the experimental and control groups of animals. However, in animals of the experimental group, this increase was more pronounced, since granulocytes and monocytes belong to the cells of innate immunity.

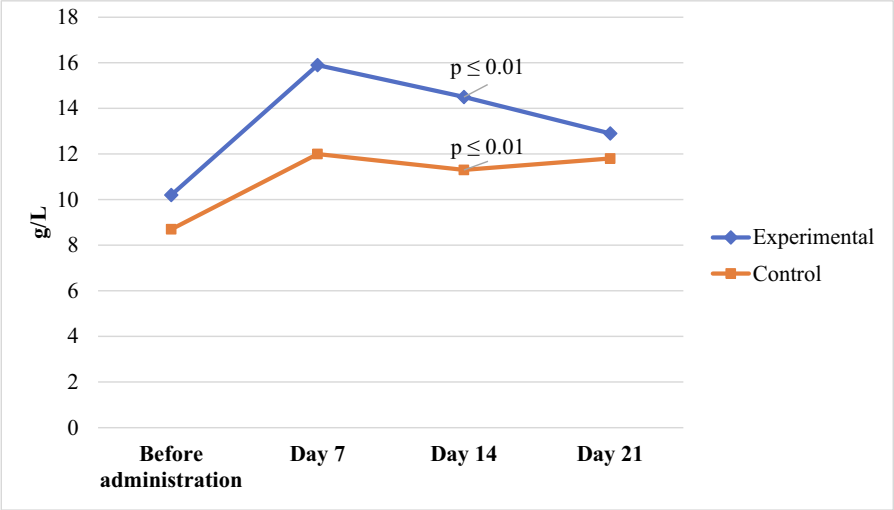


Fig. 2. Dynamics of the absolute number of granulocytes, g/L

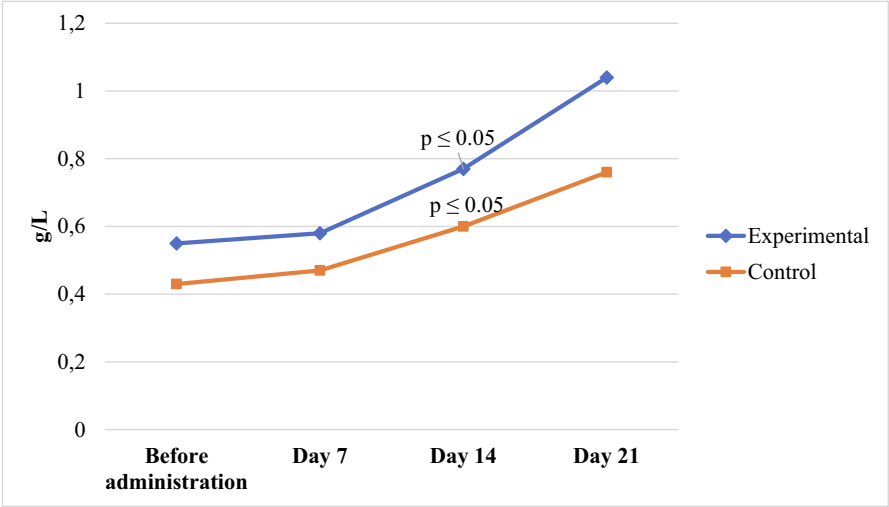


Fig. 3. Dynamics of the absolute number of monocytes, g/L

The analysis of changes in the absolute number of lymphocytes (Fig. 4) showed that the number of these cells in animals of the control and experimental groups almost did not differ, that is, both its decrease at the beginning of the experiment and its subsequent increase as well as a further increase on the 7th, 14th, and 21st days.

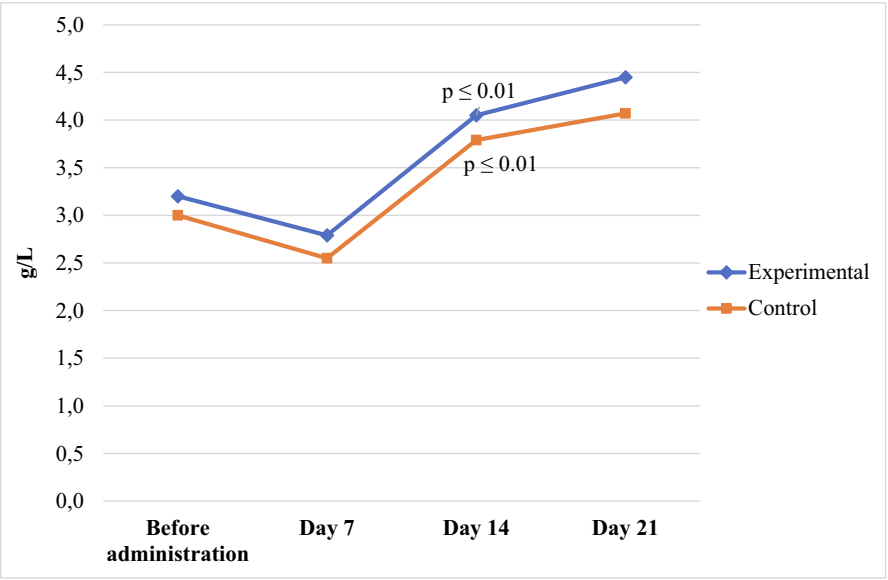


Fig. 4. Dynamics of the absolute number of lymphocytes, g/L

Taking into account the fact that T cells belong to the cells of adaptive immunity, it can be assumed that there are no specific receptors on the surface of cell membranes during administration of the humic biologically active feed additive Humilid. The effect of Humilid is manifests through the more archaic links of the body's immune defense.

Analyzing the dynamics of indicators that characterize the erythroid lineage, namely, the erythrocyte count, the hemoglobin content, and the hematocrit index, we emphasize that no reliable changes were found (Table 5).

At the same time, a tendency towards a decrease in the population of blood eosinophils in the experimental animals was determined. At the same time, the number of these cells, on the contrary, increased in animals of the control group. The opposite reaction was observed in relation to platelets count in blood of experimental animals. In dynamics, the population of these cells in the control group decreased, and in the experimental group, on the contrary, it increased within physiological limits.

Table 5

The dynamics of the number of formed blood elements, hemoglobin content and hematocrit in puppies under the influence of Humilid, a humic-based biologically active additive (M ± m; n = 3)

Parameters	Before		Day 7		Day 14		Day 21	
	E	C	E	C	E	C	E	C
Erythrocyte, 10^9/L	5.783 ± 0.115*	6.223 ± 0.219*	5.263 ± 0.114	6.017 ± 1.485	5.78 ± 0.104	6.12 ± 0.901	5.557 ± 0.439	5.767 ± 0.167
Eosinophils, 10^9/L	3.367 ± 1.501	4.933 ± 2.540	2.8 ± 0.346	4.567 ± 0.404	2.733 ± 0.808	5.2 ± 0.693	2.367 ± 0.482	5.367 ± 1.501
Platelets, 10^9/L	385.667 ± 79.097	317.0 ± 10.392	336.333 ± 83.716	243.333 ± 75.056	535.000 ± 109.119	282.667 ± 161.658	656.333 ± 120.666*	409.333 ± 49.652*
Hemoglobin, g/L	122.667 ± 4.041	128.0 ± 5.196	110.667 ± 1.155	125.667 ± 30.60	123.667 ± 4.619	128.667 ± 18.475	119.667 ± 10.97	118.667 ± 2.877
Hematocrit, %	41.067 ± 1.443	42.867 ± 1.27	36.967 ± 0.981	41.167 ± 10.335	40.6 ± 1.212	42.233 ± 5.947	39.1 ± 3.637	39.533 ± 0.924

Note: 1.* $p < 0.01$; ** $p < 0.001$ compared with indicators before administration.

2. E – experimental; C – control.

Thus, the inclusion of the biologically active feed additive Humilid in the main diet of domestic animals does not cause sensitization of the body to the active ingredient of this drug. On the contrary, the inclusion of Humilid in the main diet of domestic animals has a desensitizing effect, as evidenced by a decrease in the number of blood eosinophils in the experimental animals. The main quantitative changes in blood cells under the influence of Humilid are related to the cellular innate immunity, as evidenced by an increase in the total number of granulocytes and monocytes.

5. Dynamics of Hematological Parameters In Dogs after the Application of Fosprenil, the Biologically Active Compound with Immunotropic Action

In the first stage of study, the authors determined the main indicators of immunograms and hematological parameters of experimental and control animals, as well as the degree of individual sensitivity to immunologic agents. It was found that the relative lymphocyte count, as well as the phagocytic activity of neutrophils were within physiological limits. It was noted that the relative number of T cells in animals of the experimental and control groups tended to increase, and the number of NK cells – decreased. After determining the individual sensitivity to immunologic agents (by the percentage of inversion of activated T cells) it was found that the optimal (inversion of more than 10%) it is to the drug Fosprenil.

The next stage of study included comparing the state of immunoreactivity of the organism by changes in immunogram parameters in the experimental and control groups in the dynamics after a 5-day course of Fosprenil (Table 6).

It was found that after Fosprenil administration the number of lymphocytes in animals of the experimental group decreased by 3.7% ($p < 0.01$), while in control animals, a decrease in this indicator was not observed. In experimental animals, there was also a decrease in the number of T cells by 6% ($p < 0.001$), and this occurred only due to cells with helper activity by 5.36% ($p < 0.001$), and the number of cells with cytotoxic (suppressor) activity, on the contrary, it tended to increase slightly (by 0.9%). Such changes in the relative number of subpopulations of T cells indicate the immunoregulatory effect of the study drug, which was expressed by an increase in the level of antiviral protection (an increase in T suppressor cells). The T lymphocyte subpopulation in the control group did not change significantly.

By contrast to T cells, the B lymphocyte count increased by 3.3% ($p< 0.001$) in animals of the experimental group, while in the control group – only by 0.6%. When Fosprenil administred to animals of the experimental group, the relative number of B cells increased by 2% ($p < 0.01$) compared with control animals.

Table 6

Dynamics of the relative number of immunocompetent cells after Fosprenil administration, (M ± m; n = 4)

Parameters	Before drug administration		After drug administration	
	Control	Experimental	Control	Experimental
Lymphocytes, %	26.00 ± 7.67	30.3 ± 9.61	26.6 ± 3.51	26.6 ± 1.15*
T cells, %	74.00 ± 9.16	73.3 ± 14.05**	73.3 ± 8.32	67.3 ± 9.45
T helper cells, %	56.60 ± 7.57	56.0 ± 14.0**	56.6 ± 4.6	50.66 ± 10.2
T suppressor cells, %	17.34 ± 5.00	16.6 ± 1.15	17.66 ± 4.3	17.3 ± 1.15
B cells, %	12.00 ± 2.46	11.3 ± 2.02	12.6 ± 2.05	14.6 ± 3.16**
NK cells, %	5.00 ± 1.73	3.3 ± 0.85	5.0 ± 1.0	6.6 ± 1.52*
Phagocytic activity of neutrophils, g/L	46.0 ± 12.16	44.0 ± 5.2	49.3 ± 7.57	54.6 ± 11.01**

Note: *$p < 0.05$; **$p < 0.01$ compared with animals of the control group.

In dogs injected with Fosprenil, there was an increase in the number of broad-plasma lymphocytes (NK cells) by an average of 3.3% ($p < 0.01$), and in animals of the control group, this figure remained unchanged. It was found that in animals of the experimental group after administration of the drug phagocytic activity of neutrophils increased by 6.3% ($p < 0.001$) compared with controls.

When studying the changes in the sensitivity of T cells to the immunologic agent before and after its introduction, a significant decrease in the activity of surface receptors of T cells was observed, which was expressed in a decrease in the percentage of inversion.

In addition to the specific immune response, which is provided mainly by macrophages and lymphocytes, a large number of other cells and substances non-specifically affect the completeness and adequacy of immune responses. Some of the non-specific factors of the immune response were studied by us simultaneously with the indicators of immunograms (Table 7).

Table 7

Dynamics of hematological parameters after Fosprenil administration

(M ± m; n = 4)

Parameters	Before drug administration		After drug administration	
	Control	Experimental	Control	Experimental
Erythrocyte, 10^9/L	5.97 ± 0.76	5.44 ± 0.78	6.94 ± 0.79	7.57 ± 1.88**
Hemoglobin, g/L	126.0 ± 18.9	118.0 ± 19.3	157.3 ± 21.5	177.3 ± 42.9***
Hematocrit, %	42.23 ± 5.87	37.9 ± 6.3	49.36 ± 6.43	54.46 ± 13.78
Monocytes, %	0.73 ± 0.15	0.80 ± 0.05	0.60 ± 0.10	0.87 ± 0.15
Eosinophils, %	2.33 ± 0.06	2.63 ± 0.57	2.16 ± 0.095	1.6 ± 0.056
Platelets, g/L	451.7 ± 89.4	457.3 ± 49.3	485.67 ± 48.3	406.0 ± 76.3

Note: *$p < 0.05$; **$p < 0.01$ compared with animals of the control group.

Experiments showed a more pronounced increase in the absolute number of erythrocytes by 2.13 g/L ($p < 0.01$) in animals of the experimental group, while in dogs of the control group this increase occurred, on average, by only 0.97 T/L ($p < 0.01$). Together with the increase in the number of erythrocytes, a more pronounced increase in the hemoglobin content was noted in animals of the experimental group ($p < 0.001$).

When analyzing blood platelets, no reliable changes were found, although they are active participants in immune reactions and carry antigens and immune complexes, possess pronounced cytotoxic properties. Therefore, during the use of immunotropic agents in the recommended doses, the sensitivity of lymphocytes to these drugs decreases. At the same time, hematological indicators change: the number of erythrocytes, hemoglobin content, hematocrit and the relative number of eosinophils. The dynamics of these changes should be taken into account when conducting courses of immunity correction for various clinical conditions in small animal veterinary practice.

6. Influence of Cycloferon on Immunophysiological Status in Puppies

The data obtained during the determination of hematological parameters in puppies before vaccination became the ground for the study. It was found that almost 80% of puppies have higher absolute and relative lymphocyte count in blood than physiological limits. Also, specific antibodies against viral diseases, whose live antigen is included in the vaccine, are also often recorded in high titers, not typical for maternal.

The aim of this study was to establish the effect of the immunomodulatory drug on the immunophysiological condition of puppies and the possibility of prescribing it as a biological agent to prevent possible adverse effects during vaccinations. There was a significant difference between the initial titer of specific antibodies against canine distemper and canine parvovirus (Table 8).

Table 8

Specific antibody titers against viral diseases in puppies before and after Cycloferon administration (n = 3)

№ animals in the experiment	Antibody titer against canine distemper, U/mL		Difference	Antibody titer against canine parvovirus, U/mL		Difference
	Before administration	After administration		Before administration	After administration	
1	11.75	14.06	+2.31	356.0	198.4	-158.4
2	14.77	16.23	+1.46	314.4	136.4	-178
3	13.16	6.92	-6.24	594.1	383.7	-210.4
M ± m	13.23 ± 1.51	12.33 ± 4.73	-1.18	421.3 ± 51.0*	239.5 ± 128.5**	-182.3

Note: *p < 0.05; **p < 0.01 between antibody titers before and after drug administration.

The antibody titer against canine distemper, which was on average 13.23 ± 1.51 U/mL, was significantly lower by 31.8 times ($p<0.05$) than antibody titers against canine parvovirus (421.3 ± 51.0 U/mL).

Cycloferon administration resulted in a decrease in specific immunoglobulin titers against canine distemper in puppies' blood by an average of 1.18 U/mL, and

against canine parvovirus – by 182.3 U/mL ($p < 0.01$) in 14 days. Therefore, specific antibody titers against both diseases were significantly reduced. In this case, an increase in absolute and relative number of B cells was observed as a compensatory reaction in 14 days after drug administration (Table 8).

Table 9 shows the absolute content of white blood cells, lymphocytes and their regulatory subpopulations before and after the administration of Cycloferon – an immunotropic agent.

Table 9

Absolute content of white blood cells, lymphocytes and their subpopulations before and after Cycloferon administration (M ± m; n = 3)

Parameters		Before administration	After administration
Cell count, g/L	Leukocytes	7.50 ± 1.41	7.30 ± 1.36*
	Lymphocytes, %	3.63 ± 1.23	4.90 ± 1.26**
	T cells, %	2.66 ± 1.53	3.48 ± 1.13*
	T helper cells, %	2.20 ± 1.28	3.11 ± 1.09**
	T suppressor cells, %	0.46 ± 0.18	0.41 ± 0.10
	B cells, %	0.38 ± 0.12	0.69 ± 0.26
	NK cells, %	0.45 ± 0.18	0.49 ± 0.15
	Phagocytic activity of neutrophils, g/L	3.01 ± 1.04	1.03 ± 0.10*
Immunoregulatory index (Th/Ts)		5.30 ± 1.12	7.40 ± 1.03**

Note: * $p < 0.01$; ** $p < 0.001$ compared with indicators before drug administration.

It was found that after Cycloferon dministration, there was an increase in absolute number of lymphocytes by 34.99% ($p < 0.001$) in puppies. At the same time, the absolute number of white blood cells decreased by 2.67% ($p < 0.01$). In the dynamics, a significant increase in the absolute number of T cells before and after drug administration by 30.71% ($p < 0.01$) was observed. The absolute number of T helper cells after drug administration increased by 41.36% ($p < 0.001$) and T suppressor cells,

on the contrary, significantly reduced by 10.61% ($p < 0.01$). The above-mentioned quantitative changes in T cell subpopulations were also reflected in the immunoregulatory index. The ratio of T helper cells to T suppressor cells increased by 39.62% ($p < 0.001$) after Cycloferon administration.

The analysis of the phagocytic activity of neutrophils revealed a significant decrease by 65.83% ($p < 0.01$) in 14 days after the administration of the immunotropic agent. This effect may be associated with the activation of immunoregulatory mechanisms in the body of puppies and, as a consequence, the entry of harmful metabolites into blood due to the normalization of metabolic processes.

Against the background of decreasing phagocytic activity of neutrophils, there was an increase in the number of natural killer cells, the absolute number of which before the drug administration was 0.454 ± 0.179 g/L and after 14 days – 0.490 ± 0.148 g/L. Exactly NK cells play a major role in the body's antiviral defence and destroy tumor cells and cells infected with the virus.

Analysis of the relative number of lymphocytes and their regulatory subpopulations under Cycloferon treatment (Table 10) also showed an increase in lymphocyte count by an average of 17% and a decrease in the number of neutrophils capable of phagocytosis by 34% ($p < 0.01$). The loading test of activated T cells E-RFL (T cells forming rosettes with sheep erythrocytes) with retinal neuroantigen in experimental animals in the dynamics after treatment with an immunologic agent showed that this figure decreased by 4 times ($p < 0.001$).

According to the obtained data, the degree of sensitization of the organism to the antigen of the herpes simplex virus after 14 days of observation increased by 118.5% ($p < 0.001$). This may be due to an increase in the absolute and relative numbers of T cells in the puppies.

Comparing the data of loading tests before and after Cycloferon administration, an increase in the sensitivity of activated T cells to interferon in 2.9 times ($p < 0.001$).

Thus, Cycloferon administration to puppies contributes to a decrease in specific antibody titers and an increase in absolute and relative number of B cells. After this

drug administration, the absolute and relative lymphocyte count increased, but the number of phagocytic neutrophils decreased.

Table 10

Relative indicators of cellular immunity after Cycloferon administration, % (M ± m; n = 3)

Parameters	Before administration	After administration
Lymphocytes	49.00 ± 18.08	66.00 ± 12.74
T cells	75.00 ± 9.45	71.00 ± 1.15
T helper cells	63.00 ± 12.22	63.00 ± 1.15
T suppressor cells	13.00 ± 3.05	8.70 ± 1.15
B cells	11.00 ± 1.15	13.00 ± 3.05
NK cells	8.00 ± 0.07	10.3 ± 2.15
Phagocytic activity of neutrophils	78.00 ± 2.00	44.7 ± 15.00*
Inversion to the herpes virus	7.00 ± 2.74	15.30 ± 5.03**
Inversion to retinal antigens	8.00 ± 2.46	2.0 ± 0.01**
Inversion to interferon	3.00 ± 1.16	8.70 ± 2.16**

Note: * $p < 0.01$; ** $p < 0.001$ compared with values before drug administration.

However, the application of Cycloferon increased the receptor activity in lymphocytes, which was manifested in an increase in the percentage of inversion to the herpes antigen and interferon in 2.2 and 2.9 times, respectively, while sensitization to retinal neuroantigens, in contrast, decreased.

The obtained data should be used in small animal veterinary practice in order to prevent complications after the introduction of biological stimuli (vaccines) during vaccinations.

7. Dynamics of Indicators of Immunophysiological Status in Puppies Under Exposure to Biological Stimulation

Immunity is a clearly defined system of interaction in the organism, formed as a result of evolution. Anti-infective defence of the animal body as a phenomenon is logically embedded in modern views about immunity because it is an integral part of it. In recent decades, the number of dogs vaccinated in veterinary clinics according to the schemes recommended by manufacturers has increased significantly. But most vaccines administred to animals are of imported origin. Schemes that propose vaccine administration do not take into account the peculiarities of our country, one of which is the presence of a large number of stray dogs, which certainly affects the prevalence of biological stimuli [176]. This fact substantiates studying the influence of a biological stimulus on cellular immunity in order to prevent possible risks that may be caused by vaccine administration.

We performed immunological studies on ten 2-month-old puppies born from the same bitch. To study peculiarities of lymphocyte response to biological stimulation (vaccine administration) depending on their initial number, puppies were divided into two groups. The first experimental group (4 animals) included puppies that had a high number of active lymphocytes before vaccination, and the second (6 animals) – with a low number of lymphocytes.

Table 11 shows the absolute number of lymphoid cells and their immunoregulatory subpopulations in blood of puppies before and after biological stimulation (introduction of Duramune Max 5/4 vaccine).

It was found that the absolute number of leukocytes in animals of the 1st group before biological stimulation significantly differed from this indicator in puppies of the 2nd group and was 8.70 ± 0.36 g/L. After 14 days in these animals, the level significantly decreased to 7.30 ± 0.43 g/L ($p < 0.05$). In experimental animals of the 2nd group, it became significantly higher after vaccine administration by 59.05% ($p < 0.05$).

Table 11

Absolute number of blood leukocytes, lymphocytes and their regulatory subpopulations in puppies before and after biological stimulation (M ± m)

Parameters	1st group (n = 4)		2nd group (n=6)	
	Before vaccination	After vaccination	Before vaccination	After vaccination
Leukocytes, g/L	8.70 ± 0.36	7.30 ± 0.43*	5.25 ± 0.93	8.35 ± 1.01*
Lymphocytes, g/L	3.71 ± 0.38	2.03 ± 0.09**	2.08 ± 0.32	3.17 ± 0.09**
T cells, g/L	2.95 ± 0.55	1.63 ± 0.08*	1.64 ± 0.234	2.22 ± 0.01*
T helper cells, g/L	2.34 ± 0.38	1.20 ± 0.06*	1.33 ± 0.117	1.65 ± 0.06*
T suppressor cells, g/L	0.61 ± 0.06	0.43 ± 0.05*	0.32 ± 0.02	0.58 ± 0.06*
B cells, g/L	0.40 ± 0.10	0.19 ± 0.01**	0.19 ± 0.01	0.35 ± 0.07**
NK cells, g/L	0.35 ± 0.06	0.20 ± 0.01*	0.20 ± 0.03	0.35 ± 0.04*
Phagocytic activity of neutrophils, g/L	2.06 ± 0.54	2.01 ± 0.46*	1.87 ± 0.08	2.58 ± 0.54
Immunoregulatory index (Th/Ts)	3.90 ± 0.74	3.00 ± 0.91	4.30 ± 0.40	3.00 ± 0.35*

Note: intragroup difference is significant at * $p < 0.05$; ** $p < 0.01$.

As can be seen from Table 11, a decrease in the absolute lymphocyte content by 45.28% ($p < 0.01$) was observed in animals of the 1st group after biological stimulation, and in the 2nd group, on the contrary, this indicator increased by 52.40% ($p < 0.01$).

However, comparing the effect of a biological stimulus on a subpopulation of T cells, it is obvious that the antiproliferative (inhibitory) effect on lymphoid blood cells is more pronounced than stimulating. The difference in the absolute number of T cells before and after vaccine introduction in animals of the 1st group decreased by 44.77% ($p < 0.01$) and increased by 35.03% group, ie antiproliferative processes prevailed over the process of proliferation cells 2.35 times.

The same dynamics was observed when determining the number of T helper and T suppressor cells (Table 11). If before vaccine administration the absolute content of T helper cells was 2.34 ± 0.38 g/L in animals of the 1 st group, then this indicator

significantly decreased by 48.63% ($p < 0.05$) after the introduction. After vaccine administration in puppies of the 2nd group, the absolute number of T helper cells increased and reached 1.65 ± 0.06 g/L, while before introduction its indicator was lower by 24.34% ($p < 0.05$).

It was found that under the influence of a biological stimulus, the dynamics of the number of T suppressor cells is similar to T helper cells in puppies of both groups. Thus, before vaccine introduction in animals of the 1st experimental group, the absolute number of T suppressor cells was 0.61 ± 0.06 g/L, and it decreased by 30.33% ($p < 0.05$) in 14 days. In the 2nd group, the absolute count of T suppressor cells increased by 80.56% ($p < 0.05$) after exposure to a biological stimulus.

A decrease in the absolute number of T helper cells and T suppressor cells in puppies of the 1st experimental group and an increase of these figures in the 2nd experimental group led to a significant equalization of the immunoregulatory index (T helper cells to T suppressor cells ratio) in experimental animals.

In the previous section, it was noted that puppies with an immunoregulatory index within 3.0 had an adequate immune response during a spontaneous violation of the immunophysiological status. When the value of the immunoregulatory index was 3.90 ± 0.74 before vaccine administration in animals of the 1st group and 4.30 ± 0.90 in the 2nd group after vaccine administration, it decreased by 23.08% in the 1st and by 30.23% in the 2nd experimental group ($p < 0.01$).

A decrease in the absolute number of phagocytic cells under biological stimulation was established in animals of the I group by 2.18% ($p < 0.01$), while in puppies of the II experimental group this indicator increased by 38.02% ($p < 0.01$).

Indicators of humoral immunity status (absolute and relative number of B cells, content of specific antibodies in blood serum) are presented in the table. 11–13. It was established that the animals of the 2nd group, which before exposure to the biological stimulus (Table 11) had a lower absolute number of B cells (0.19 ± 0.01 g/L), after vaccination were characterized by an increase in this indicator by 83.68% ($p < 0.01$). In puppies, in which the absolute number of B cells was initially higher (0.40 ± 0.10 g/L), its decrease by 51.86% ($p < 0.01$) was observed after vaccine administration.

These data may indicate an immunomodulatory effect of a biological stimulus on B cell immunity in puppies.

Table 12 presents the dynamics of the titer of specific immunoglobulins G (IgG) against the main viral diseases in carnivores. The titer of specific antibodies against canine distemper virus and canine parvovirus in animals of the 1st group was slightly higher in contrast to the puppies of the 2nd experimental group, but this fact had no significant effect on the synthesis of antibodies. In both groups, after biological stimulation, an increase in their titer was noted. In the 1st group, there was a probable increase in the titer of antibodies against canine distemper virus by 34.44% ($p < 0.05$). In the 2nd experimental group, despite the lower initial level of immunoglobulins (10.60 ± 2.63 U/mL), the titer increased by an average of 20 U/mL or 188.68% ($p < 0.05$).

Table 12

Dynamics of the titer of specific immunoglobulins G before and after biological stimulation

Group	No. of animal in the experiment	Before vaccination, U/mL		Day 14 after vaccination, U/mL		Difference, U/mL	
		Against CDV	Against CP	Against CDV	Against CP	Against CDV	Against CP
1st (n = 4)	1	24,27	60.0	39.94	356.4	+15.67	+296.4
	2	10.63	45.2	28.56	348.6	+17.93	+303.4
	3	10.37	36.58	35.52	339.2	+25.12	+302.6
	4	12.82	42.4	32.12	337.7	+19.3	+295.3
M ± m		14.52 ± 6.56*	46.05 ± 9.97*	34.04 ± 4.85	345.48 ± 8.73	+19.52	+299.4
2nd (n = 6)	1	10.55	43.67	33.31	351.1	+22.76	+307.4
	2	7.77	36.75	24.76	394.2	+16.99	+357.45
	3	12.32	46.0	29.06	365.4	+16.74	+319.4
	4	7.54	27.4	35.52	336.1	+27.98	+308.7
	5	14.36	22.45	28.78	355.7	+14.42	+333.25
	6	11.19	17.31	32.18	321.2	+20.99	+303.89
M ± m		10.6 ± 2.63*	32.26 ± 11.7*	30.6 ± 3.84	353.95 ± 25.8	+20.0	+321.7

Note: 1. *Intragroup difference within one titer of specific antibodies is significant at $p < 0.05$.

2. CP – canine parvovirus; CDV– canine distemper virus.

When analyzing the titer of immunoglobulins G against canine parvovirus before the action of a biological stimulus, it was established that this indicator in both groups is higher than the titer of immunoglobulins G against canine distemper virus.

After vaccine administration, the dynamics of the titer of specific IgG against canine parvovirus in animals was also characterized by a significant increase in animals of both experimental groups. In the 1st group, before biological stimulation, this indicator increased on average by 650.22% ($p < 0.001$), and in animals of the 2nd group – by 997.18% ($p < 0.05$). The increase in dynamics in animals of the 1st group, where the initial titer of IgG against canine parvovirus was higher, occurred by 299.4 U/mL, while in dogs of the 2nd group, with a lower initial titer, it was by 321.7 U/mL, i.e. by 22.3 U/mL (7.45%) more.

When analyzing the relative lymphocyte count and their regulatory subpopulations before and after biological stimulation, it was established that the initial lymphocyte count in animals of the 1st experimental group was higher by 9.09% ($p < 0.001$) compared with the representatives of the 2nd experimental group (Table 13). In 14 days after biological stimulation, this indicator decreased by 14% ($p < 0.001$) in the 1st experimental group, and by only 2% ($p < 0.05$) in the 2nd experimental group.

Before vaccine administration in both experimental groups, the relative T lymphocyte count was the same. After 14 days, the biological stimulus caused an increase in this indicator by 1% ($p < 0.01$) in animals of the 1st group, and a decrease by 9% ($p < 0.01$) – in the 2nd group.

A decrease in the relative number of T helper cells and an increase in T suppressor cells were observed in animals of both experimental groups.

Thus, after vaccine administration, the relative number of T helper cells in animals of the 1st experimental group decreased by 4% ($p < 0.05$) with a simultaneous increase in the relative number of T suppressor cells by 4.5% ($p < 0.05$). At the same time, in dogs of the 2nd group, these changes were, respectively, 13.0% ($p < 0.01$) and 2.7% ($p < 0.05$).

The relative count of phagocytizing neutrophils in the puppies of the 2nd experimental group was lower than the average in animals of the 1st group by 2.0% ($p < 0.01$).

After vaccine administration, there were significant changes in the phagocytic activity of neutrophils. In animals of the 1st group, this indicator increased by only 1%,

which was insignificant. In representatives of the 2nd group, it was found to decrease by 8% ($p < 0.01$).

Table 13

The relative count of lymphocytes and their regulatory subpopulations before and after the introduction of a biological stimulus (M ± m), %

Cell count, %	Animal group			
	1st (n = 4)		2nd (n = 6)	
	Before vaccination	After vaccination	Before vaccination	After vaccination
Lymphocytes	44.00 ± 7.07	30.00 ± 7.93*	40.00 ± 6.21	38.00 ± 8.78*
T cells	78.00 ± 1.63	79.00 ± 6.83**	78.00 ± 9.75	69.00 ± 11.7**
T helper cells	63.00 ± 4.42	59.00 ± 6.21**	63.00 ± 9.26	51.00 ± 8.54**
T suppressor cells	16.50 ± 1.91	21.00 ± 5.29**	15.00 ± 2.09	17.70 ± 3.8**
B cells	11.00 ± 2.58	9.50 ± 2.31**	9.00 ± 2.42	11.80 ± 1.63*
NK cells	9.50 ± 1.0	10.00 ± 1.63*	9.70 ± 2.33	11.00 ± 1.51
Phagocytic activity of neutrophils	41.00 ± 7.74	42.00 ± 9.29	59.00 ± 7.11	51.00 ± 9.0**

Note: intragroup difference is significant at * $p < 0.05$; ** $p < 0.01$.

In animals of the 2nd experimental group, where the activity of cells was lower compared with animals of the 1st group, vaccine administration contributed to an increase in the number of active cells. At the same time, the relative number of active T suppressor cells increased by only 2.7% ($p < 0.01$) against 4.5% ($p < 0.01$) in animals of the 1st group. The absolute count of active B cells increased almost twice in animals of the 2nd group, and the relative count – only by 2%.

It should be noted that in animals of the 2nd group this decrease was more significant, although the titer of synthesized specific antibodies in response to biological stimulation was higher.

It can be assumed that the higher specific immunoglobulin content in blood, the lower the level of sensitivity of lymphocytes to a certain antigen.

Therefore, the analysis of the immunoreactivity in puppies depending on the initial count of immunocompetent cells under biological stimulation allows us to predict the dynamics of changes in indicators of cellular and humoral immunity. It must be said that the immunomodulating effect of a biological stimulus will depend not only on the initial count of immunocompetent cells, but also on the degree of stress in puppies' organism, sufficient oxygen supply, and the cascade of oxygen-dependent immunological reactions. Taking into account the fact that a decrease in specific antibody titers in puppies was observed already in the second week of life, their number at the time of vaccination should be minimal. The presence of a high specific antibody titer in blood before vaccination may indicate the persistence of an immunogen in the organism, and the corresponding hypothesis should be taken into account when introducting a biological stimulus [177].

8. Indicators of the Cellular Immunity in Dogs under the Influence of Membrane Plasmapheresis

There are two main methods for plasmapheresis – gravity and filtration. The first one is carried out by blood centrifugation with a constant or intermittent blood flow in special devices PF-05, PF-3-05, FK-3.5 or foreign production by Gambro, Fresenius, Cobe, Dideco, Terumo or in bags (vials) in centrifuges of RS-6, OS-6, CL-3.5 type. The second method is based on blood filtration in special plasma filters.

The basis for conducting this study was the data on the successful use of filtration plasmapheresis for the purpose of correcting the immune and other systems of the organism, namely, removing the pressure of metabolic products on the main links of cellular and humoral immunity. The author suggests to carry out 2–4 sessions with an interval of 1–2 days for optimal sanitation of the internal environment of the body. With this substitution of plasma only with an isotonic solution, there are no significant changes in homeostasis components (proteins, fats, and carbohydrates). The cellular and humoral elements of homeostasis formed after the plasmapheresis procedure are

free from metabolic pressure and retain their main functional purpose for a longer period.

Table 14 shows the dynamics of the absolute count of leukocytes, lymphocytes and their T subpopulations during plasmapheresis. As research has shown, after the 1st procedure, the absolute leukocyte count in blood from animals of the experimental group decreased by 3.08 g/L or 26.29% ($p < 0.01$).

Table 14

Dynamics of the absolute count of leukocytes, lymphocytes and their T subpopulations during plasmapheresis (M ± m; n = 3)

Parameters	Before the 1st procedure		Before the 2nd procedure		Before the 3rd procedure	
	Animal groups					
	E	C	E	C	E	C
Leukocytes, g/L	10.65 ± 2.48	10.93 ± 2.05	7.85 ± 0.26**	10.33 ± 2.80*	9.40 ± 0.81**	10.4 ± 2.75
Lymphocytes, g/L	1.59 ± 0.21	1.53 ± 0.07	2.24 ± 0.08	1.63 ± 0.23	2.23 ± 0.13	1.64 ± 0.06
T cells, g/L	1.07 ± 0.03	1.09 ± 0.19	1.27 ± 0.27	1.12 ± 0.09	1.46 ± 0.25	1.08 ± 0.16
T helper cells, g/L	0.66 ± 0.02	0.57 ± 0.02	0.80 ± 0.07**	0.59 ± 0.07	1.01 ± 0.28**	0.61 ± 0.03
T suppressor cells, g/L	0.41 ± 0.18	0.44 ± 0.03**	0.48 ± 0.10	0.45 ± 0.05	0.45 ± 0.04**	0.45 ± 0.09
Immunoregulatory index (Th/Ts)	2.80 ± 0.08	2.27 ± 0.59	2.60 ± 0.07	2.07 ± 0.08	3.00 ± 0.07**	2.23 ± 0.08

Note: 1. *$p < 0.05$; **$p < 0.01$ compared with animals of the control group.

2. E – experimental; C – control.

In animals of the control group, a decrease of only 0.6 g/L or 5.49% ($p < 0.05$) was established. The 2nd procedure of plasmapheresis in animals of the experimental group contributed to an increase in the absolute leukocyte count to 9.4 ± 0.81 g/L ($p < 0.01$) in comparison with the indicators after the 1st procedure. At the same time, the indicator remained unchanged in the control (10.4 ± 2.75 g/L). Before the plasmapheresis procedure, the absolute lymphocyte count in the control and experimental groups did not differ significantly and was within physiological limits.

Evaluation of cellular immunity indicators in dogs after one plasmapheresis procedure showed that the animals of the experimental group had a decrease in the absolute leukocyte counts by 35% ($p < 0.01$) compared with the control group. In particular, after the 1st procedure, an increase in this indicator by 40.88% ($p<0.01$) was observed, and after the 2nd, there were no significant changes in this cell population.

The animals of the experimental group demonstrated a pronounced increase in the absolute count of T cells with helper activity by 20.79% ($p < 0.05$) after the 1st procedure and further by 26.50% after the 2nd procedure.

At the same time, in the control group, the dynamics of this indicator was 2.27% after the 1st procedure and 4.78% after the 2nd. There were no significant changes in the absolute count of T cells with suppressive activity in animals of the experimental group. Thus, after the 1st procedure, a tendency towards an increase of this indicator by 16.05% was noted, and after the 2nd, a significant decrease in this indicator by 6.25% occurred again ($p < 0.05$).

An increase in the absolute count of T helper cells and a decrease in T suppressor cells led to a significant increase in the immunoregulatory index by 7.14% ($p < 0.01$) in animals of the experimental group after the 1st procedure, followed by an increase of 15.38% ($p < 0.01$) after the 2nd procedure.

It should be noted that the immunoregulatory index was within physiological limits during plasmapheresis procedures. This suggests that plasmapheresis does not cause an imbalance in T lymphocyte subpopulations, and, accordingly, does not disrupt the ability of these cells to provide an adequate immune response. As we established earlier, the ratio between immunoregulatory subpopulations of lymphocytes with T helper and T suppressor activity turned out to be the most valuable for practice [184]. Violation of this ratio can be one of the risk factors for the development of immunopathological reactions after plasmapheresis.

Table 15 shows the dynamics of absolute count of B cells, NK cells and phagocytizing neutrophils during plasmapheresis. The absolute count of B cells (state of humoral immunity) in blood increased by 25.42% ($p < 0.01$) in animals of the experimental group after the 1st procedure and by 25.68% ($p < 0.05$) after the 2nd

procedure. In dogs of the control group, this indicator was significantly lower compared with the experimental dogs before and after the 2nd procedure.

The increase in B ymphocyte count (on average by 25%; $p < 0.01$) was more significant compared with the absolute T lymphocyte count (by 19%; $p < 0.05$). A possible factor that caused this effect is probably the removal of a significant amount of immunoglobulins from the body during plasmapheresis and it was their reduction that resulted in the proliferation of these cells. The most significant decrease in the absolute count of phagocytizing neutrophils by 47.14% ($p < 0.05$) was in dogs of the experimental group after the 1st plasmapheresis procedure. After the 2nd repeat plasmapheresis, this indicator increased and constituted 3.68 ± 0.71 g/L.

Table 15

Dynamics of the absolute count of B cells, NK cells and phagocytizing neutrophils during plasmapheresis, g/L (M ± m; n = 3)

Parameters	Before the 1st procedure		Before the 2nd procedure		Before the 3rd procedure	
	Animal groups					
	E	C	E	C	E	C
B cells	0.18 ± 0.04	0.16 ± 0.02*	0.22 ± 0.07	0.17 ± 0.01	0.28 ± 0.03	0.18 ± 0.05**
NK cells	0.14 ± 0.03	0.14 ± 0.01	0.27 ± 0.02	0.13 ± 0.03	0.25 ± 0.01	0.14 ± 0.03
Phagocytic activity of neutrophils	4.46 ± 0.16	3.77 ± 0.12	2.36 ± 0.18*	3.94 ± 0.94	3.68 ± 0.71	3.87 ± 0.64

Note: 1. *p < 0.05; **p < 0.01 compared with animals of the control group.

2. E – experimental; C – control.

Therefore, the comparative analysis of immunograms performed during plasmapheresis procedures in dogs allowed to determine that several indicators by which cellular immunity is evaluated change significantly. The dynamics of these changes should be taken into account when performing plasmapheresis for various clinical conditions in practice of small domestic animals.

9. Indicators of Immunity In Puppies under the Influence of Trifuzol in the Form of a Liposomal Emulsion during Bivalent Vaccine Administration

It was established that immunophysiological indicators have multidirectional dynamics. Table 16 shows the dynamics of the absolute count of lymphocytes and their immunoregulatory subpopulations. Thus, in the puppies of the control group, the absolute lymphocyte count decreased from 2.75 [2.6–3.0] to 2.55 [2.4–2.7] g/L after vaccine administration.

Table 16

Dynamics of the absolute count of lymphocytes and their immunoregulatory subpopulations during vaccine administration, median [minimum-maximum]

Parameters	Before introduction		After introduction	
	Control (n = 4)	Experimental (n = 4)	Control (n = 4)	Experimental (n = 4)
Lymphocytes, g/L	2.75 [2.6–3.0]	2.45 [2.3–2.6]*	2.55 [2.4–2.7]	2.75 [2.7–3.1]*
T cells, g/L	1.6 [1.5–1.9]	1.5 [1.4–1.7]	1.5 [1.3–1.7]	1.85 [1.7–3.0]*
T helper cells, g/L	1.25 [1.0–1.4]	1.2 [1.0–1.3]*	1.15 [0.9–1.3]	1.5 [1.3–1.7]
T suppressor cells, g/L	0.4 [0.3–0.5]	0.35 [0.3–0.5]*	0.35 [0.2–0.5]	0.3 [0.2–0.5]*
B cells, g/L	0.4 [0.3–0.5]	0.35 [0.3–0.4]	0.4 [0.3–0.6]	0.45 [0.4–0.5]*
Immunoregulatory index (Th/Ts)	3.8 [3.6–4.0]	3.15 [3.0–3.4]*	3.35 [3.0–3.5]	3.85 [3.8–4.2]*

Note: *$p < 0.05$; **$p < 0.01$ compared with animals of the control group.

Comparing this indicator in puppies of the experimental group, an increase from 2.45 [2.3–2.6] g/L to 2.75 [2.7–3.1] g/L was noted. The dynamics of a decrease in the control and an increase in the experimental group were also established regarding the absolute count of T cells and their subpopulations. At the same time, this tendency is less pronounced in T suppressor cells and more – in T helper cells. Analyzing the

absolute count of B cells, it was established that the number of these cells in the dynamics slightly decreased in the control group by 4%, and in the experimental group, its significant increase was registered by 7% ($p < 0.06$).

A decrease in the immunoregulatory index was noted 14 days after vaccination in control animals and an increase in this index – in the experimental group.

The dynamics of indicators of the phagocytic activity of neutrophils and NK cell count are shown in Table 17.

Table 17

Dynamics of indicators of phagocytic activity of neutrophils and NK cell count, median [minimum-maximum], g/L

Parameters	Before introduction		After introduction	
	Control (n = 4)	Experimental (n = 4)	Control (n = 4)	Experimental (n = 4)
Phagocytic activity of neutrophils	2.25 [2.0–2.5]	2.5 [2.3–2.8]*	2.4 [2.2–2.6]	2.1 [2.0–2.4]*
NK cells	0.2 [0.1–0.3]	0.2 [0.2–0.4]	0.2 [0.2–0.3]	0.2 [0.2–0.4]*

Note: *$p < 0.05$; **$p < 0.01$ compared with animals of the control group.

Table 17 provides data on the change in the absolute count of phagocytizing neutrophils in puppies of the experimental and control groups.

It was established that, in contrast to the dynamics of lymphocytes, the phagocytic activity of neutrophils increased from 2.25 [2.0–2.5] to 2.4 [2.2–2.6] g/L (ie by 12%) in puppies of the control group. At the same time, this figure reliably decreased by 10% in animals of the experimental group.

Analyzing the dynamics of the absolute NK cell count (Table 17), it should be noted that there were practically no significant quantitative changes in the population of these cells in puppies of the control and experimental groups. However, the range of dynamics narrowed from 1.0–3.0 to 2.0–3.0 in puppies of the control group.

Ambiguous results were obtained when determining the titer of specific antibodies (IgG) against canine parvovirus and canine distemper in puppies in the experimental and control groups in the dynamics (Table 18).

Table 18

Dynamics of the titer of specific antibodies (IgG) against canine parvovirus and canine distemper, median [minimum-maximum], U/mL

Parameters	Before administration		After administration	
	Control (n = 4)	Experimental (n = 4)	Control (n = 4)	Experimental (n = 4)
Titer of antibodies against canine parvovirus	7.3 [6.5–8.0]	6.0 [5.0–7.0]	21.0 [18.0–24.0]	0.0 [0.0–0.0]*
Titer of antibodies against canine distemper	15.0 [12.0–17.0]	6.0 [5.0–8.0]*	27.0 [20.0–32.0]	0.0 [0.0–0.0]*

Note: *$p < 0.05$ compared with animals of the control group.

It was established that before vaccine administration in animals of the experimental and control groups, the titer of specific antibodies against canine parvovirus was relatively low and did not differ significantly. A 1.85 times higher titer of specific antibodies against canine distemper was noted before vaccine administration in puppies of the control group. Analyzing the titer of antibodies 14 days after vaccine administration, it was established that the puppies of the control group had a significant increase in this indicator. Thus, the titer of IgG against canine parvovirus increased by 3.65 times ($p < 0.06$), and the titer of IgG against canine distemper increased by 2.2 times ($p < 0.06$). In puppies of the experimental group, the titer of IgG against canine parvovirus and canine distemper virus decreased to zero after 14 days.

10. Dynamics of Indicators of Cellular Immunity in Dogs after the Administration of Methisazone and Albuvir in the Form of a Liposomal Emulsion

Taking into account the stress in dogs during visits to clinical veterinary institutions and the lack of skills in injecting drugs among animal owners, it is urgent to develop methods that will reduce the number of visits and ensure the prolongation of the effect of the immunotropic agent. The purpose of this research section was to study the efficiency of immunity correction using biologically active substances with immunotropic action in the form of liposomal emulsions. The research was conducted on three groups of animals, one of which was a control group. Animals of the 1st experimental group were administered Albuvir in the form of a liposomal emulsion at a dose of 1 mL per 10 kg body weight, twice, with an interval of 48 hours. The animals of the 2nd experimental group were administered Methisazone in the form of a liposomal emulsion at a dose of 1 mL per 10 kg of body weight, twice, with an interval of 48 hours. Animals of the control group were administered with an isotonic solution of NaCl at a dose of 2 mL with an interval of 48 hours.

In order to evaluate the indicators of the immunophysiological status in the organism during the administration of the above-mentioned drugs, blood samples were taken from dogs of all three groups before drug administration and before each subsequent administration.

Studies of immunograms in dogs before drug administration showed that the initial absolute indices in the experimental and control groups differed somewhat (Table 19). Thus, the absolute leukocyte count was 5.63 ± 0.37 g/L in the control, which was less by 34.99% than in animals of the 1st experimental group, but more than 15.99% ($p<0.01$) in comparison with the representatives of the 2nd experimental group. At the same time, it should be emphasized that the absolute count of lymphocytes and their subpopulations, as well as the immunoregulatory index before the beginning of drug administration, were the highest in animals of the 2nd experimental group.

Table 19

The count of leukocytes, lymphocytes and their subpopulations in canine blood before the administration of biologically active substances (M ± m; n = 3)

Parameters	Absolute count, g/L			Relative count, %		
	C	1st group	2nd group	C	1st group	2nd group
Leukocytes	5.63 ± 0.37	7.6 ± 1.35*	4.73 ± 2.20	–	–	–
Lymphocytes	1.94 ± 0.41	3.61 ± 1.04*	2.11 ± 0.55	34.33 ± 5.85	46.00 ± 10.39	39.3 ± 11.11
T cells	1.04 ± 0.09	2.44 ± 0.62	1.61 ± 0.12	54.66 ± 8.32	70.66 ± 15.14	76.0 ± 4.00
T helper cells	0.70 ± 0.15	1.84 ± 0.54	1.04 ± 0.07*	36.66 ± 7.02	51.33 ± 4.16	54.0 ± 11.13
T suppressor cells	0.34 ± 0.07	0.61 ± 0.15	0.57 ± 0.06*	18.0 ± 5.29	19.33 ± 6.28	22.66 ± 3.31
Immunoregulatory index (Th/Ts)	2.20 ± 0.40	4.10 ± 0.85	3.06 ± 0.79*	–	–	–

Note: 1. *p < 0.01 compared with animals of the control group.

2. C – control group; 1st group – Albuvir; 2nd group – Methisazone.

A greater number of cells in animals of the 2nd experimental group compared with the control was noted against the background of a decrease in the count of phagocytizing neutrophils (Table 20) both in absolute count (by 41.35%; $p < 0.01$) and in relative count (by 17.33% ; $p < 0.01$).

The absolute and relative count of NK cells is slightly higher, and a decrease in the intensity of phagocytic activity was noted in both experimental groups compared with the control.

Analyzing the absolute and relative indicators of immunograms before drug administration, it should be noted that the count of T helper cells was higher in both experimental groups. The absolute count of B cells as an indicator of the state of the humoral immunity turned out to be higher in animals of the 1st experimental group, and the relative count – in the 2nd experimental group.

Table 20

The count of B cells, NK cells and phagocytizing neutrophils in canine blood before the administration of biologically active substances (M ± m; n = 3)

Parameters	Absolute count, g/L			Relative count, %		
	C	1st group	2nd group	C	1st group	2nd group
B cells	0.25 ± 0.01	0.46 ± 0.03	0.25 ± 0.09	13.33 ± 3.05	12.00 ± 2.21	20.66 ± 5.01
NK cells	0.15 ± 0.03	0.35 ± 0.07	0.23 ± 0.08*	7.66 ± 2.08	9.33 ± 1.15	11.00 ± 3.00
Phagocytic activity of neutrophils	2.07 ± 0.36*	1.46 ± 0.32	1.01 ± 0.22*	56.66 ± 12.85	39.33 ± 11.38	40.66 ± 10.26

Note: 1. *p < 0.01 compared with animals of the control group.

2. C – control group; 1st group – Albuvir; 2nd group – Methisazone.

Already after the first introduction of biologically active substances in the form of liposomal emulsions (Table 21), changes in immunogram indicators were noted, which were expressed by an increase in leukocyte count in both experimental groups; moreover, they were more significant in animals administered Methisazone: 42 vs. 12% (p < 0.01).

At the same time, in animals of both experimental groups, a synchronous decrease in lymphocyte count by 36% compared with the initial values was observed (Table 22). It should be noted that the count of T helper cells decreased more in the dogs of the 1st experimental group, and T suppressor cells – in the 2nd experimental group, and this was reflected in the immunoregulatory index, which increased more than twice in the 2nd experimental group (p < 0.01).

Regarding NK cells, a single administration of Albuvir reduced their count, and Methisazone, on the contrary, resulted in an increase of this figure by 60% (p < 0.01) compared with the initial values. NK cells are a population of lymphoid cells that do not have the characteristics of T and B cells. Their participation in the immune response

consists in the organization of a direct cytotoxic response to malignantly transformed and virus-infected cells [178]. A single administration of Methisazone caused a more pronounced increase in the number of phagocytizing neutrophils than Albuvir administration.

Table 21

The number of leukocytes, lymphocytes and their subpopulations in canine blood after the first administration of biologically active substances in the form of liposomal emulsions (M ± m; n = 3)

Parameters	Absolute count, g/L			Relative count, %		
	C	1st group	2nd group	C	1st group	2nd group
Leukocytes	6.46 ± 0.72	8.53 ± 2.82	6.73 ± 0.35*	–	–	–
Lymphocytes	2.54 ± 0.58	2.30 ± 0.56	1.35 ± 0.32	39.00 ± 3.80	32.33 ± 8.57	20.00 ± 5.10
T cells	1.03 ± 0.09	1.56 ± 0.09	1.00 ± 0.05	45.30 ± 5.70	65.33 ± 16.77	73.30 ± 6.11
T helper cells	0.77 ± 0.12	1.03 ± 0.25	0.75 ± 0.05*	30.30 ± 3.23	46.0 ± 12.0	51.33 ± 14.46
T suppressor cells	0.35 ± 0.05	0.523 ± 0.04	0.25 ± 0.02*	13.00 ± 3.11	19.33 ± 14.46	22.00 ± 16.37
Immunoregulatory index (Th/Ts)	2.60 ± 0.60	3.30 ± 0.61	3.46 ± 0.12	–	–	–

Note: 1. *p < 0.01 compared with animals of the control group.

2. C – control group; 1st group – Albuvir; 2nd group – Methisazone.

Table 22 shows the count of B cells, NK cells and phagocytizing neutrophils after the first administration of biologically active substances in the form of liposomal emulsions. A significant increase in the absolute count of phagocytic neutrophils was established in animals administered metisazone (2nd experimental group) – by 119.31% (p < 0.01) after the first drug administration. In animals of the 1st experimental group, this indicator also significantly increased by 15.58% (p < 0.01) after Albuvir administration. Along with this, the absolute count of B cells in both

experimental groups had a tendency to decrease, which in animals of the 2nd experimental group (Methisazone) was 94% ($p < 0.01$) against 44% in dogs of the 1st group.

Table 22

The number of B cells, NK cells and phagocytizing neutrophils in canine blood after the first administration of biologically active substances in the form of liposomal emulsions (M ± m; n = 3)

Parameters	Absolute count, g/L			Relative count, %		
	C	1st group	2nd group	C	1st group	2nd group
B cells	0.23 ± 0.04	0.32 ± 0.09*	0.13 ± 0.01	9.66 ± 2.30	15.0 ± 4.08	9.66 ± 2.21
NK cells	0.16 ± 0.02	0.25 ± 0.003	0.38 ± 0.04	6.3 ± 1.15	10.66 ± 1.52	12.0 ± 1.73
Phagocytic activity of neutrophils	1.65 ± 0.48	1.69 ± 0.40	2.22 ± 0.52*	57.33 ± 12.74	28.66 ± 5.03	41.33 ± 9.01

Note: 1. *$p < 0.01$ compared with animals of the control group.

2. C – control group; 1st group – Albuvir; 2nd group – Methisazone.

Repeated drug administration to experimental animals caused changes in immunological indicators, which were mainly related to lymphocytes. However repeated drug administration had almost no effect on leukocyte count (Table 23). Three days after repeated treatment, the lymphocyte count (due to T suppressor cells and B cells) continued to decrease by 15% ($p < 0.01$) in animals that were administered Albuvir, and the number of T helper cells slightly increased compared with the condition after the first troduction. An increase in the absolute number of T suppressor cells to 0.95 ± 0.06 g/L and relative to 30.6 ± 9.3% after the second administration of Methisazone led to a significant decrease in the immunoregulatory index to 1.68 ± 0.14 ($p < 0.01$).

Table 23

The number of blood leukocytes, lymphocytes and their subpopulations in dogs after repeated administration of biologically active substances in the form of liposomal emulsions (M ± m; n = 3)

Parameters	Absolute count, g/L			Relative count, %		
	C	1st group	2nd group	C	1st group	2nd group
Leukocytes	6.20 ± 1.40	8.33 ± 2.22	6.23 ± 1.84*	–	–	–
Lymphocytes	2.35 ± 0.40	1.96 ± 0.26	2.82 ± 0.13	30.90 ± 9.10	24.00 ± 7.00	44.60 ± 8.10
T cells	1.03 ± 0.16	1.66 ± 0.32	2.12 ± 0.37*	44.20 ± 8.70	82.66 ± 9.20	74.00 ± 5.20
T helper cells	0.72 ± 0.11	1.30 ± 0.25	1.17 ± 0.08	28.49 ± 4.60	65.30 ± 5.03	43.30 ± 9.40
T suppressor cells	0.34 ± 0.59	0.35 ± 0.11	0.95 ± 0.06*	14.40 ± 2.50	16.60 ± 4.10	30.60 ± 9.30
Immunoregulatory index (Th/Ts)	2.10 ± 0.50	4.30 ± 1.13	1.68 ± 0.14*	–	–	–

Note: 1. *p < 0.01 compared with animals of the control group.

2. C – control group; 1st group – Albuvir; 2nd group – Methisazone.

In dogs administred with Methisazone (2nd group), the recovery of the number of lymphocytes to the initial level was observed, mainly due to T suppressor cells, the number of which increased by 3.8 times ($p < 0.01$). It should be noted that the subpopulation of T suppressor cells includes cells that have not only suppressive, but also cytotoxic activity. Therefore, such an increase in cell data in our study is probably more related to a regulatory effect than to immunosuppression. Repeated administration of Methisazone caused a decrease in the number of phagocytizing neutrophils, in contrast to animals that were administered Albuvir and in which, on the contrary, this indicator increased by 34% ($p < 0.01$). It is generally known that a decrease in the phagocytic activity of neutrophils usually reflects the degree of toxic suppression of immunocompetent cells circulating in blood, or is an indicator of the beginning of active processes related to phagocytosis, which reflects the active phase of elimination from the body of metabolic products.

Table 24 shows the absolute and relative numbers of B cells, NK cells and phagocytic activity of neutrophils after repeated administration of biologically active substances in the form of liposomal emulsions.

In the animals of the 2nd experimental group, for an increase of 2% ($p < 0.01$) in the absolute number of B cells as an indicator of humoral immunity, there was a decrease in the absolute number of phagocytic cells by 4% ($p<0.01$).

Table 24

The number of B cells, NK cells and phagocytic neutrophils in canine blood after repeated administration of biologically active substances in the form of liposomal emulsions (M ± m; n = 3)

Parameters	Absolute count, g/L			Relative count, %		
	C	1st group	2nd group	C	1st group	2nd group
B cells	0.26 ± 0.01	0.26 ± 0.23	0.33 ± 0.11*	10.80 ± 2.20	14.00 ± 3.20	11.60 ± 0.57
NK cells	0.17 ± 0.02	0.22 ± 0.07	0.32 ± 0.02	7.40 ± 1.60	11.30 ± 0.57	11.00 ± 1.00
Phagocytic activity of neutrophils	2.05 ± 0.30	2.58 ± 0.39	1.33 ± 0.27*	58.40 ± 12.40	38.60 ± 9.01	37.30 ± 8.08

Note: 1. *$p < 0.01$ compared with animals of the control group.

2. C – control group; 1st group – Albuvir; 2nd group – Methisazone.

In our study, the decrease in the phagocytic activity of neutrophils was accompanied by the activation of the cytotoxic effect of T suppressor cells and NK cells, which is probably a compensatory reaction to the active influx of metabolites into the bloodstream and is regulated by signaling cytokines in a receptor-mediated way.

Therefore, the analysis of the state of the immunoreactivity of the body after the introduction of biologically active substances in the form of liposomal emulsions allows us to state that with certain changes in the immunophysiological status of the body, in which proliferative cellular processes are detected, a decrease in the activity of humoral factors and natural antiviral protection in the body in order to correct immunoregulatory mechanisms is appropriate use of biologically active substances in the form of liposomal emulsions, in particular Albuvir and Methisazone.

Liposomal emulsions, in addition to structural components and main biological substances with immunotropic action, include fat-soluble vitamins A and E.

Fig. 5 presents data on changes in vitamin E concentration in blood serum of dogs after the application of biologically active substances in the form of a liposomal emulsion.

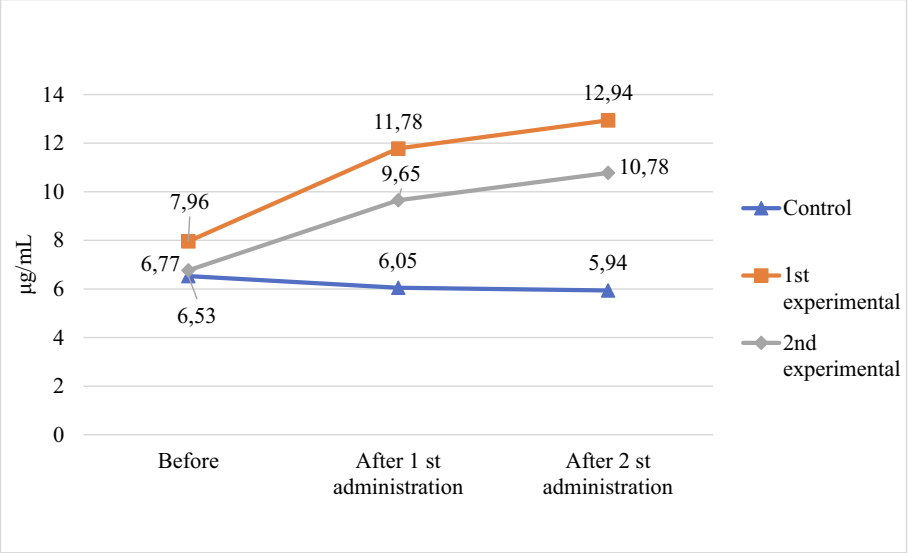


Fig. 5. Dynamics of serum vitamin E content in dogs during liposomal emulsion application

From the presented data, it can be seen that in the control group during the experiment, the concentration of this vitamin tended to decrease slightly. Thus, at the initial amount of 6.53 µg/mL, the decrease occurred to 5.94 µg/mL, the difference was 0.59 µg/mL, which is not significant. The assessment of this indicator in both experimental groups showed a tendency towards a significant increase in vitamin E content. It should be noted that in animals of the 1st group the increase occurred by 4.98 µg/mL, and in the 2nd group – by 4.01 µg/mL. After the first injection of liposomal emulsion, a more pronounced increase in the content of this vitamin in blood serum was observed than after the second.

The results of determining the content of vitamin A in blood serum of dogs after the introduction of liposomal emulsion are presented in Fig. 6.

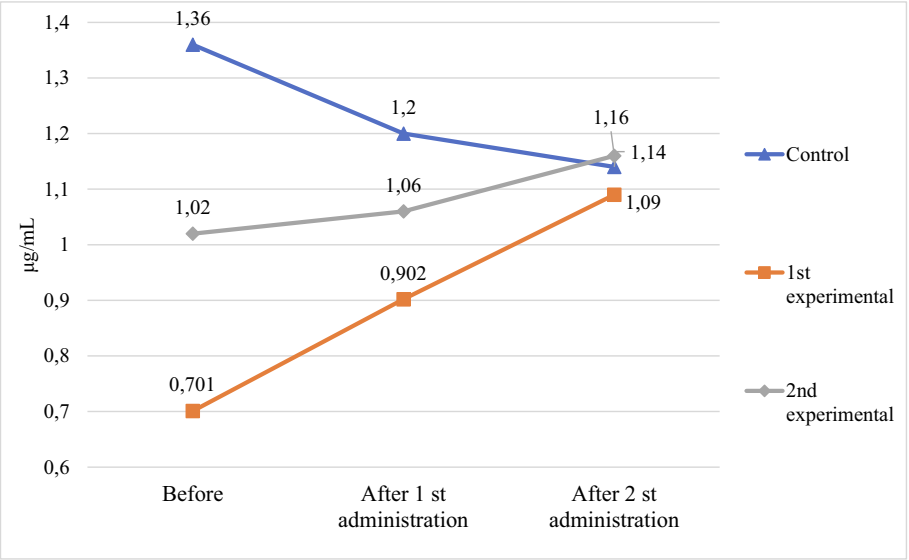


Fig. 6. Dynamics of serum vitamin A content in dogs during liposomal emulsion application

A tendency to decrease this vitamin in the blood serum of animals of the control group was also observed, as well as in the content of vitamin E. Our research established that in the 1st experimental group during the experiment, namely during the two-time administration of the liposomal emulsion, there was a more pronounced increase in the content of vitamin A from 0.701 to 1.09 µg/mL, i.e. by 0.381 µg/mL. At the same time, in animals of the 2nd experimental group, from 1.02 to 1.14 µg/mL (by 0.12 µg/mL). Considering that the most reactogenic cells of adaptive immunity are the population of T cells, namely their subpopulations of T helper cells and T suppressor cells, and the immunoreactivity of cellular immunity will depend on their adequate interaction (immunoregulatory index), we separately present the dynamics of the number of these cells in the blood plasma for the introduction of biologically active substances in the form of a liposomal emulsion.

Fig. 7 shows the influence of biologically active substances on the dynamics of the number of T cells with helper activity in the blood plasma of dogs after the introduction of liposomal emulsion. Compared with the control, the investigated drugs probably had an antiproliferative effect after the 1st administration, which was manifested in a significant decrease in the absolute number of these cells.

However, repeated administration was accompanied by an increase in the number of this cell subpopulation.

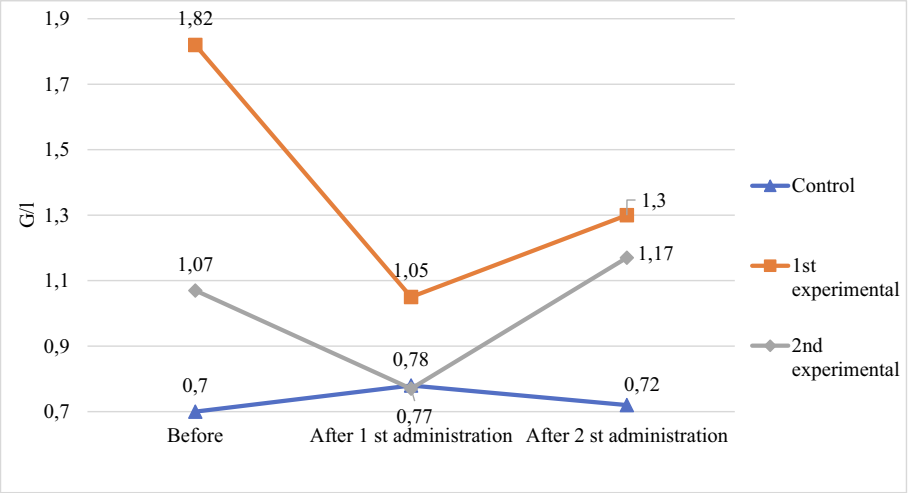


Fig. 7. Dynamics of the number of T helper cells in canine blood during liposomal emulsion application

Similar dynamics after the introduction of liposomal emulsion were also observed when assessing the number of T cells with suppressor activity (Fig. 8).

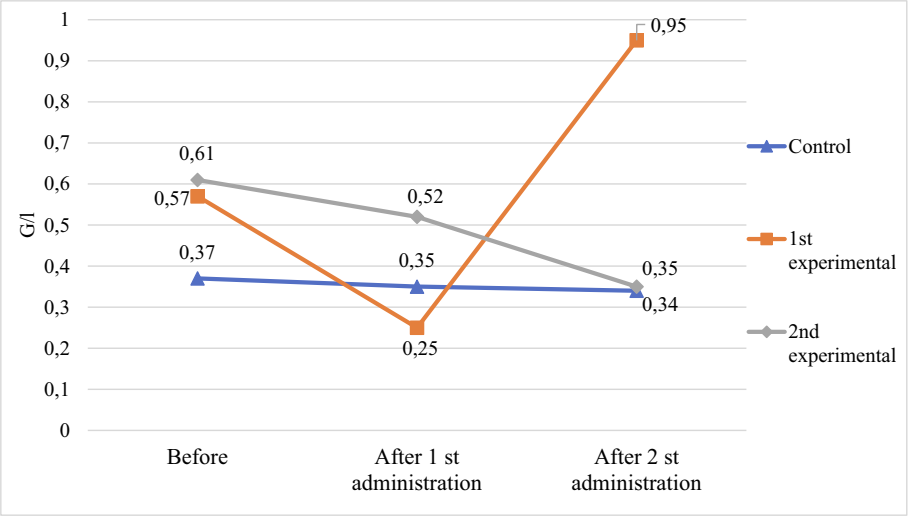


Fig. 8. Dynamics of T suppressor cells in canine blood during liposomal emulsion application

Thus, a single drug administration was accompanied by a decrease in T suppressor cells in animals of both experimental groups.

After repeated drug administration, this effect continued in animals of the 1st experimental group (administred Albuvir), and in the 2nd experimental group, a significant increase in the number of T cells with suppressor activity was observed.

Therefore, on this subpopulation of immunocompetent cells, biologically active substances with immunotropic action, included in the composition of the liposomal emulsion, affected differently, which should be taken into account when they are prescribed for the purpose of correction.

Other, equally important indicators of adaptive immunity are the number of NK cells and the phagocytic activity of neutrophils (Fig. 9).

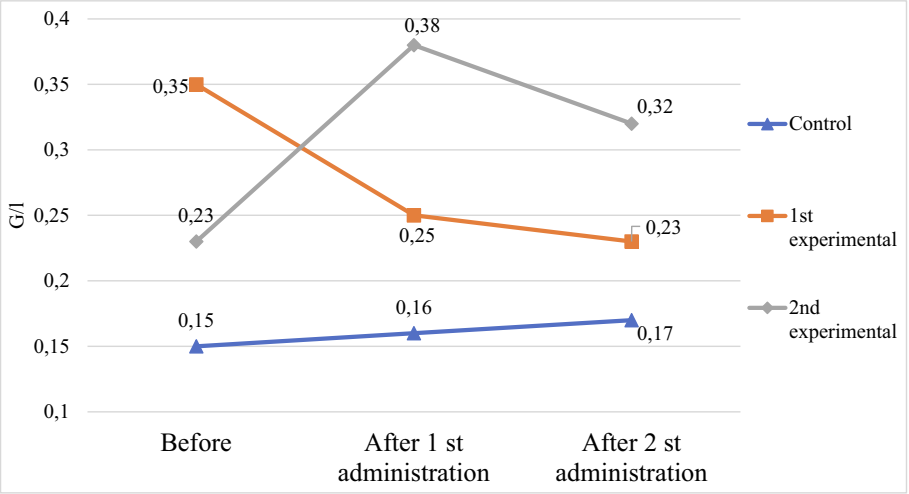


Fig. 9. Dynamics of NK cells in canine blood during liposomal emulsion application

When analyzing the dynamics of NK cells after the introduction of liposomal emulsion, results similar to the dynamics of T cells with suppressive activity were observed in animals of the first experimental group. That is, a probable antiproliferative effect was noted, which was expressed by a decrease in the number of these cells during the experiment. In the animals of the 2nd experimental group (Methisazone), a single administration of the drug was accompanied by a sharp increase in the number of NK cells, but with repeated administration, a tendency to decrease was observed.

Fig. 10 shows the dynamics of the number of neutrophils capable of phagocytosis after the introduction of liposomal emulsion.

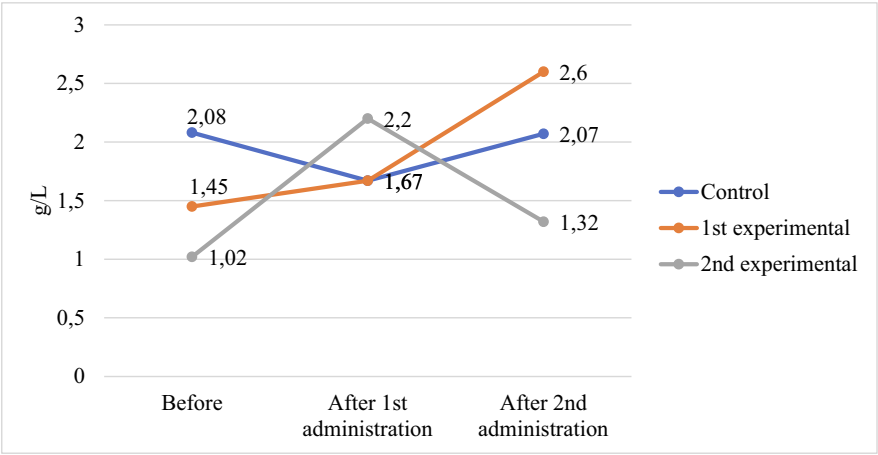


Fig. 10. Dynamics of phagocytic activity of neutrophils in canine blood during liposomal emulsion application

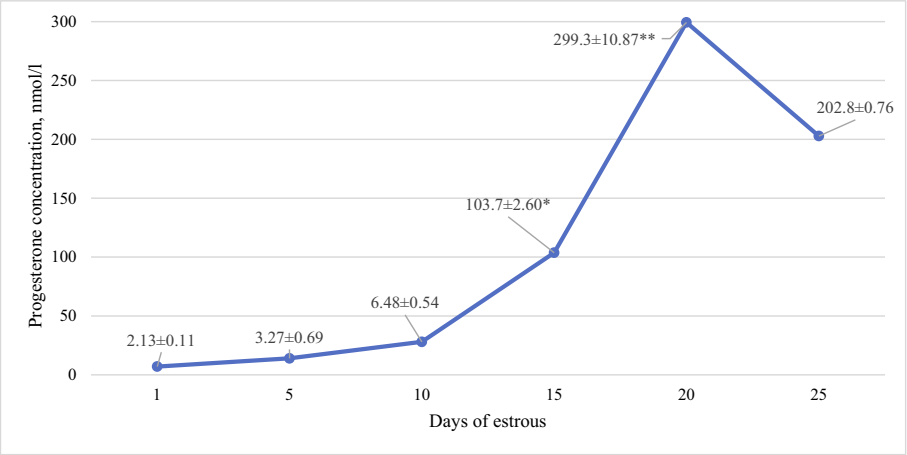
In contrast to cytotoxic cells (T suppressor and NK cells), the dynamics of phagocytic activity of neutrophils was characterized by the fact that after double Albuvir administration, this indicator gradually increased. A significant increase in the phagocytic activity of neutrophils was initially noted during a single administration of Methisazone in a liposomal emulsion (2nd experimental group). Repeated administration caused a decrease in this indicator. It should also be noted that the initial values of the phagocytic activity of neutrophils differed and, probably, this also affected the specificity of the dynamics of the indicator of neutrophil activity before phagocytosis. Therefore, the introduction of biologically active substances in the form of a liposomal emulsion has an immunomodulating effect of a different nature, which makes it possible to use these drugs for the purpose of correcting the immunophysiological status in dogs.

11. Dynamics of progesterone, estradiol, cortisol, triiodothyronine and indicators of adaptive immunity concentrations in bitches during estrus

Eight Labrador bitches aged 3 to 5 years were involved in the experiment. The animals were kept in one cattery and fed with dry feed. Infectious diseases were excluded from the animals' studied. Serum and blood plasma collected from the lateral subcutaneous vein of the forearm on days 1, 5, 10, 15, 20, 25 of the estrus were used for the study. The first day of the cycle was considered the day when bloody discharge appeared from the bitch's vagina. To obtain serum, blood was transferred into vacuum tubes of Vacutest® (Italy) with a blood coagulation activator (SiO2) followed by centrifugation at 1500 rpm. within 10 min. To obtain plasma, part of the collected blood was transferred to a test tube with EDTA. The content of estradiol, progesterone, cortisol and triiodothyronine were determined in the blood serum. The absolute content of leukocytes, lymphocytes, monocytes, neutrophils and the phagocytic activity of neutrophils (PAN) were determined in the stabilized blood.

Units of measurement of hormone content in blood serum are nmol/l. Physiological limits of progesterone content in bitches during anestrus < 0.5-6.0; in the follicular phase – 10.0-80.0; luteal phase – 25.0-60.0. Physiological limits of estradiol content in bitches during anestrus <0.073; luteal phase – 0.073-0.22; follicular phase > 0.22. Physiological limits of cortisol content in bitches are 25-250, triiodothyronine content is 0.5-2.8 (physiological limits are presented according to the instructions of the test systems, provided by the manufacturer).

The analysis of the concentration of progesterone in the blood serum of bitches had regular changes during estrus (Fig. 11.). Thus, on the 1st day of the estrus, its concentration on average was 2.13±0.11, which is typical for anestrus. During the next five days, a slight increase in progesterone content was noted to 3.27±0.69.



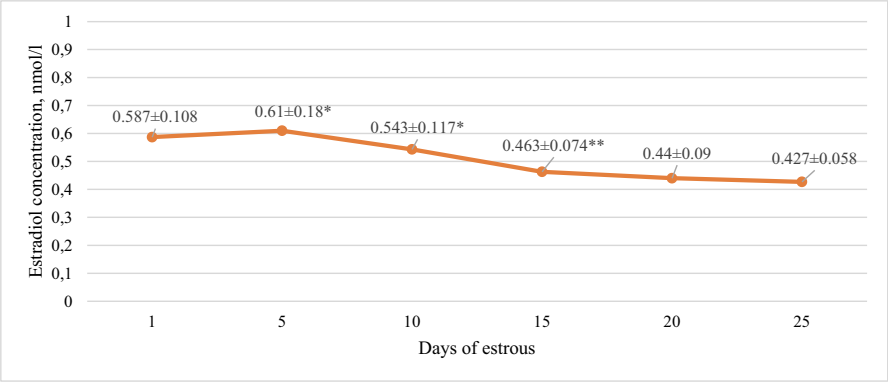
Note: * p<0.05; **p<0.001 compared to the first day of estrus

Fig. 11. Dynamics of progesterone content in blood serum during estrus

On the 10th day of estrus, the hormone content increased significantly (P≤0.05) to 6.48±0.54, this concentration is typical for the initial stage of the luteal phase of estrous cycle. Determination of progesterone concentration in blood serum on the 15th day of estrus showed a high (16-fold) significant (P≤0.05) increase compared to the 10th day. The tendency to increase the concentration was preserved in the next five days of observation and already on the 20th day, this indicator was 299.3±10.87 (P≤0.05). After the 20th day of estrus, we note a decrease in progesterone serum concentration, and already on the 25th, it decreased to 202.8±0.76.

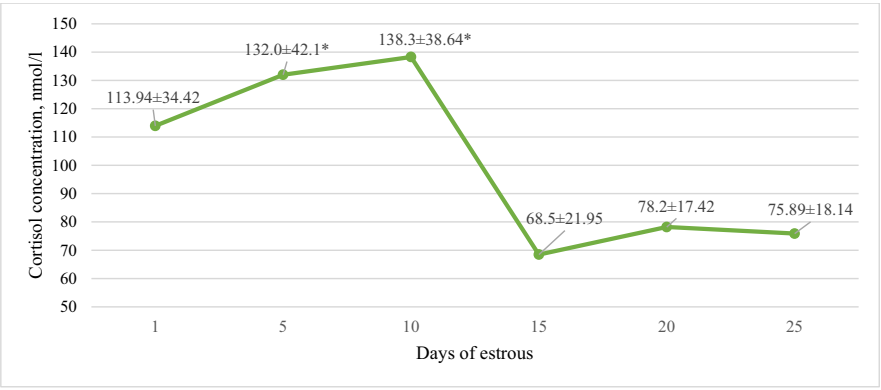
Determination of the concentration of estradiol in the blood serum of bitches during estrus showed the changes, which are shown in figure 12.

Thus, on the first day, the hormone content was 0.587±0.108, which is typical for the follicular phase of the sexual cycle. On the 5th day, an unreliable trend of increasing the concentration to 0.61±0.18 was noted, and on the 10th, a decrease to 0.543±0.117. Subsequently, with a sharp increase in progesterone concentration, the content of estradiol continued to gradually decrease and on the 15th day decreased to 0.463±0.074, on the 20th to 0.44±0.09, and on the 25th to 0.427±0.058.



Note: * p<0.05; **p<0.001 compared to the first day of estrus

Fig. 12. Dynamics of estradiol content in blood serum during estrus



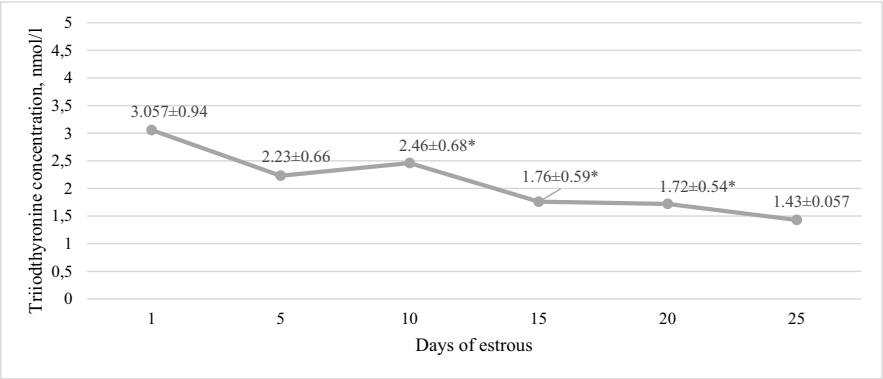
Note: * p<0.05 compared to the first day of estrus

Fig. 13. Dynamics of cortisol content in blood serum during estrus

Analysis of cortisol concentration dynamics in blood serum during estrus (Fig.13.) showed that this indicator was within physiological limits. Thus, from the 1st to the 10th day, an increase in concentration was observed from 113.94±34.42 to 138.3±38.64. Starting from the 15th day, there is a significant decrease in the concentration of this hormone in blood serum to 68.5±21.95. From the 15th to the 25th day, the cortisol content tended to increase, but within 10 nmol/l.

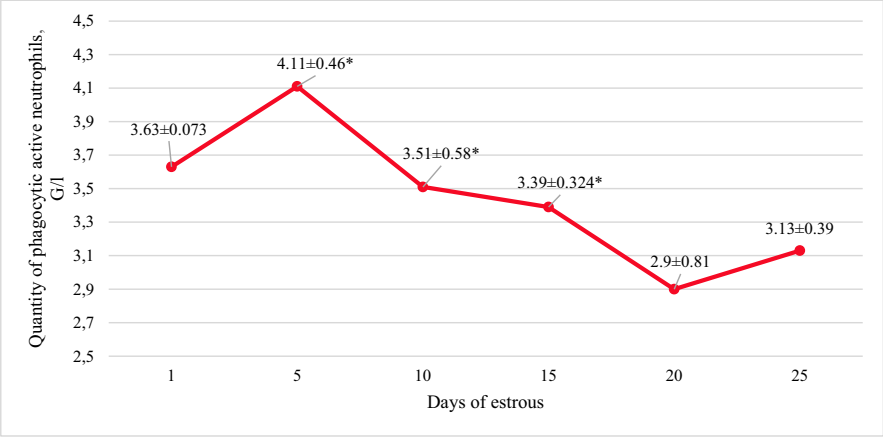
On the first day of estrus, the concentration of T3 in blood serum was 3.057±0.94, which is slightly more than physiological limits. Further determination

showed is a gradual tendency to decrease of this hormone in blood serum during estrus. A slight increase in hormone concentration is noted on the 10th day of the cycle.



Note: * p<0.05 compared to the first day of estrus

Fig.14 Dynamics of triiodothyronine content in blood serum during estrus



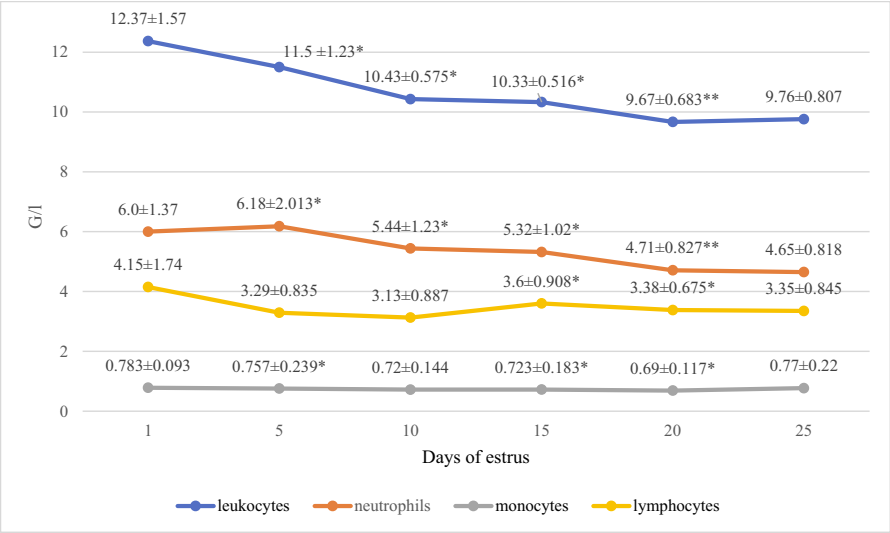
Note: * p<0.05 compared to the first day of estrus

Fig. 15. Dynamics of phagocytic activity of neutrophils during estrus

The dynamics of quantitative changes in the population of neutrophils during the experimental period are presented in figure 15.

Physiological limits of PAN in dogs vary between 55-65% of the absolute number of these cells. The ability of neutrophils to phagocytosis changed during the estrus is shown in figure 5. Thus, on the 5th day, this indicator increased significantly (p<0.05) by 13% compared to the first day and amounted 4.11±0.46 G/l. The analysis

of the subsequent dynamics shows a tendency to decrease the PAN, and in the period from 15 to 20 days, a significant decrease, up to 16%, was observed. Over the next 5 days of observation, |PAN increased from 2.9 ± 0.81 to 3.13 ± 0.39 G/l.



Note: * p<0.05; **p<0.001 compared to the first day of estrus

Fig.16. Dynamics of the absolute number of leukocytes (ANL), neutrophils, lymphocytes and monocytes during estrus

During estrus, ANL dynamics tended to decrease. So, from day 1 to day 25, this indicator decreased by 2.6 G/l (21%) (Figure 16.). However, the populations of different cells had different regularities. Thus, against the background of the general trend of ANL decreasing on 5th day, the content of neutrophils tended to increase and lymphocytes to decrease. A slight decrease in the monocyte population was also observed. On the 10th day of estrus, all the populations of the studied cells had a tendency to decrease, and on the 15th day, the number of lymphocytes increased by 13%, and the tendency of the decrease in neutrophils remained. A slight increase in ANL on the 25th day of estrus occurs against the background of monocytes.

Although the number of neutrophils had a slight (2%) tendency to decrease on the 25th day, at the same time the PAN, on the contrary, increased by 7%.

12. Analysis and Generalization of Research Results

Regulatory adaptive changes in immune reactivity are the basis of the organism's survival in a changing external environment. In different periods of ontogenesis (juvenile period, old age, and puberty) or in special cases, there are significant variations in the manifestation of mechanisms (activation of some, suppression of other), which are physiological reactions of the adaptation rather than evidence of the formation of certain pathological processes.

Changes in the immune system during ontogenesis involve a decrease or increase in the activity of various parts of the immune system in animals of different species [179]. The immune system is incredibly complex, so the demonstration of these changes relies primarily on laboratory findings.

The data on the development of the canine immune system is limited, scattered, and some older studies are difficult to access. immature innate and adaptive immune system in newborn puppy matures and acquires memory while the animal matures, and finally declines in the latter stages of life. These changes in the immune system cause different problems in the dog throughout its life and predispose the animal to various types of infections, immune-mediated diseases, and neoplasms [180].

The microbiome plays an important role in the learning and development of key components of the innate and adaptive immune systems, while the immune system manages the maintenance of key features of the host-microbe symbiosis. It is believed that in a genetically susceptible host, an imbalance in the interaction of microbiota and immunity under certain environmental conditions contribute to the pathogenesis of a variety of immune-mediated disorders [181].

In the vast majority of cases, canine diseases are caused not by one but by several etiological factors, that is, they are considered from the standpoint of a polyetiological symbiosis in combination with hypovitaminosis and mineral metabolism disorders [182], which some authors associate with the increased content of various foreign substances in the external environment. This significantly changes the genetic apparatus of many microorganisms and, according to the principle of external

communication, dramatically changes the sensitivity and strength of the immune response of animals [183].

Consequently, the development and outcome of many diseases depends on the state of the immune system. The presence of various disorders in the immune system can be the cause of secondary immunological deficiency and lead to the emergence or exacerbation of chronic bacterial and viral diseases. Often, the use of classical treatment regimens in these cases does not have the expected positive effect. The immunity correction by means of immunomodulators is a promising direction in order to increase the efficiency of etiotropic therapy and reduce the side effects that appear during their use (dysbiosis, hepatotoxicity) in secondary immunodeficiency [183].

In veterinary and human medicine, immunomodulation is an area in which extensive research was conducted for the development of methods to increase disease resistance, as well as to prevent or control host immune disorders through optimal regulation of the immune system. Today, most human and animal infectious diseases are treated and controlled predominantly with a broad spectrum antibiotics and vaccines. However, antibacterial agents are becoming increasingly ineffective due to the rapid emergence of resistant microbial strains. Thus, there is a great demand for new and improved alternative therapeutic and prophylactic strategies for the treatment of several diseases that are flaring up at an alarming rate due to increased international traffic, globalization, and changing dietary habits. Immunomodulation is focused on manipulating the immune system to control infections and other adverse health effects with precise regulation to avoid any complications [17,18, 184].

Immunomodulators affect cells that produce soluble mediators such as cytokines [185]. It should be noted that most of the existing immunomodulators are capable of influencing the immune system in one way or another. Usually, they either stimulate or inhibit the immune response [186].

Following the concept of “pharmacological interaction of drugs with immune receptors” (the p-i concept), namely that a drug can bind directly to TCR, MHC molecules, or both and thereby stimulate T cells [186, 187], it can be assumed that the efficiency of particular immunomodulators depends on the degree of sensitization of T

cells to the active ingredients of the pharmacological agent. In our experiment, studies were carried out using loading tests, where biologically active substances with immunotropic action of various origins were used as antigens. The purpose of this experiment was to establish the level of autosensitization of the dog's body to antigens, which would allow predicting the development of an immune response to the introduction of biological substances into the body. From the data obtained, it can be concluded that T cells have different activities of surface receptors. This activity determines the individual sensitivity of the organism to certain immunomodulators and, accordingly, the strength of the immune response. The administration of drugs in animals with a low level of TCR sensitivity will not entail a cascade of immunological reactions and, accordingly, a clinical effect. Excessive TCR sensitivity will develop an allergic reaction in the body after the administration of immunomodulators. Thus, drug-induced hypersensitivity reactions can develop in an organism that expresses the appropriate immunological receptors that recognize the immunomodulator as an antigen [188].

In combination, the p-i concept suggests that some drug allergies are pharmacological off-target reactions rather than true allergies. The drug is able to affect the human immune system not only as an antigen (namely, as a hapten bound to a carrier molecule) but also in a pharmaceutical way, namely, by acting on (immune) receptors [189].

Determining the level of the activity of TCR provides new opportunities for conducting tentative methods for correcting immune dysfunctions and preventing side effects the during the use of immunomodulators. The expediency of individual selection of immunocorrective therapy (taking into account the sensitivity of lymphocytes to immunomodulators) significantly increases the efficiency of treatment and was confirmed by other researchers [189].

Another factor that determines the success in the prescription of immunomodulators is the dose of the drug and the duration of its use (course). In the available literary sources, there are no data on the effect of the administration of immunologic agents in the recommended doses during their use on changes in the

individual sensitivity of the body, namely the activity of TCR.

In our studies using Fosprenil as an immunomodulator in clinically healthy dogs at the recommended dose (0.2 mL/kg) and a five-day course, a decrease in the activity of TCR to this drug was observed after the end of the course. Comparison of indicators of immunoreactivity of the organism of animals of the experimental and control groups after a five-day course of a biologically active agent of immunotropic action (Fosprenil), as well as determining changes in the individual sensitivity of the organism to this drug, the changes in the sensitivity of T cells before and after the administration of the immunologic agent was examined and the activity of TCR significantly decreased, which was expressed by a decrease in the percentage of inversion, was observed. So, this indicator decreased by 1% in animals of the control group and by 6.3% ($p < 0.001$) in experimental animals. The number of B cells doubled ($p < 0.01$) in the dogs of the experimental group, while the absolute number of these cells remained almost unchanged in the dogs of the control group. The data obtained indicate that the administration of immunomodulators at the recommended dose can affect the immune system due to a decrease in the receptor sensitivity of immunocompetent cells, and, accordingly, receive a clinical effect associated with immunological tolerance to immunomodulators. Such a clinical effect may not be long-lasting and cause re-emergence of signs of immune system dysfunction. In our opinion, one of the promising areas of research in immunology is precisely the study of the dose-dependence and duration of the application of immunomodulators on the efficiency of immunity correction.

Thus, immunomodulators includes drugs with direct immunotropic activity, which in therapeutic doses change the functions of the immune system. The essence of the phenomenon of immunomodulation is that when using a pharmacological agent in certain doses and time regimes, desired shifts in the immune system occur either as a result of the direct effect of drugs on the functional activity of immune defense cells, or as a result of changes in the interaction of immunocompetent cells or their products [190].

Maturation of the immune system occurs from birth to approximately six months

of age. Although the puppy was considered immunocompetent at 6–12 weeks of age, it is not possible to accurately predict the onset of immunocompetence as it depends on the presence of maternal antibodies. However, maternal antibodies suppress the development of the endogenous immune response in newborns and is a major obstacle to successful vaccination [191,192]. In an attempt to determine the optimal age for the beginning of the vaccination, the specific humoral immune response induced by several vaccines was investigated in puppies of different ages and breeds [193, 194].

It is believed that the antibody response to vaccination is specific for each animal and depends on the age of the dog, the titer of protective antibodies, and the type of vaccine [195].

In many countries, the issue of stray dogs, whose population lives in large enough numbers, especially in large cities, has not yet been resolved. Stray dogs are natural carriers of the main canine viruses, as well as biological objects in which viruses can change their antigenic properties and persist and be released into the environment for a long time. Given that their places of stay coincide with the places where owned dogs walk, they clearly affect the antigenic load of the body of dogs that are kept by their owners. This indicates that high titers of specific antibodies against the main viral diseases, which are found in puppies before vaccination, with a high degree of probability can be formed in the presence of a natural strain of the virus in the body.

According to literature data [196], there is no clear answer about the influence of maternal antibodies on the formation of an adaptive immune response during vaccination. It should also be noted that most veterinary hospitals are deprived of the opportunity or do not use the opportunity to determine the titer of specific antibodies against major viral diseases before vaccination. In view of the above, the drug Trifuzol from the group of 1,2,4-triazole derivatives, which affects the humoral part of adaptive immunity, in particular, a significant decrease in specific antibody titers, can be introduced in clinical veterinary medicine. In addition, the drug affects the increase in the absolute number of T and B cells in blood serum, which is also important for an adequate immune response during vaccination. The drug should be administered to puppies in a week before the expected vaccination. The number of injections is

determined directly by the veterinarian but if the drug in the form of a liposomal emulsion is applied, one subcutaneous injection is enough.

Despite widespread vaccination, viral infections in dogs remain the leading cause of their death, especially among young animals. It is largely unknown which factors are related to vaccination and which are decisive for unsuccessful vaccination [19].

According to researchers, the most important problem in the eradication of infectious diseases is unsuccessful attempts at immunization, including: a) presence of interfering maternal antibody titers; b) lack of immune system response; c) possible return to virulence [197]. The introduction of a vaccine into the body of animals, as a biological stimulus, in any case leads to changes in the immune parameters during the post-vaccination period, at the same time, vaccination can potentially lead to one of a wide range of possible adverse consequences [198]. Studies conducted by other researchers confirm that vaccination against viral infections is ineffective in Australia and indicate that veterinarians should consider underlying infections as a differential diagnosis in cases with a relevant clinical picture, regardless of the vaccination status in dogs [199].

We conducted immunological studies on ten 2-month-old puppies born from the same bitch. To study the peculiarities of the lymphocyte response to biological stimulation (vaccine administration), puppies were divided into two groups depending on their initial number. The 1st experimental group (4 animals) included puppies that had a high number of active lymphocytes before vaccination and the 2nd experimental group (6 animals) included puppies that had a low number of active lymphocytes. According to the results of these studies, it was found that polyvalent vaccine administration had an immunoregulatory effect on the cellular immunity in puppies and the development of the humoral immunity. This was manifested in the fact that the dynamics of the amount of T suppressor cells is similar to that of T helper cells in animals of both groups under exposure to a biological stimulus. Thus, before vaccine administration, the absolute amount of T suppressor cells in animals of the 1st experimental group was 0.61 ± 0.06 g/L and decreased by 30.33% ($p < 0.05$) after

14 days. In the 2nd group, the absolute amount of T suppressor cells increased by 80.56% ($p < 0.05$) after exposure to a biological stimulus.

A decrease in the absolute amount of T helper and T suppressor cells in the puppies of the 1st group and their increase in the puppies of the 2nd experimental group led to a probable equalization of the immunoregulatory index (T helper cells to T suppressor cells ratio) in the experimental animals.

Analyzing the obtained data, it should obviously be pointed out that the adequacy of the immune response to vaccine administration will depend on many factors, including the initial specific antibody titers. However, according to the authors, the vaccine itself as a biological stimulus will not cause the development of immunological complications, there is a high probability that individual characteristics of the immunophysiological state in each individual animal are directly dependent on the presence of a persistent viral infection in the body.

Physical and psychological stress should be considered as contributing factors to the development of autoimmune diseases, since numerous studies on animals and humans have demonstrated the influence of various stress factors on immune function. Moreover, many retrospective studies revealed a significant number (up to 80%) of patients reported unusual emotional distress prior to disease onset [200].

Fear and anxiety-related behaviors are common in domestic dogs and can trigger a physiological stress response in humans who are exposed to things they perceive as causing fear or anxiety. The stress response is associated with a number of changes in hormonal and immune modulation and, as has been proved, is associated with disease processes and reduced lifespan in many species. It was predicted that dogs with fear and anxiety disorders would have a shorter lifespan and increased frequency and severity of the disease [201]. Research results indicate that how an owner "behaved" or felt about their dog was positively correlated with lifespan ($R^2 = 0.18$, $p < 0.001$). Dogs with extreme antisocial fear and separation anxiety were found to have an increased severity and incidence of skin disease ($R^2 = 0.03$, $p < 0.001$). Although neither fear of strangers nor any of the other fear or anxiety scales was associated with specific causes of death, fear of strangers was associated with significantly reduced

lifespan ($R^2 = 0.16$, $p < 0.001$). There are data that suggest that stress associated with fear or anxiety disorder can have a negative impact on the health and lifespan of domestic dogs [201].

In this regard, the involvement of drugs of biological origin, natural regulators that are able to perform physiologically adequate correction of violations of the immunological reactivity in the body and to modulate the adaptive mechanisms of immune homeostasis, also seems promising in the veterinary practice [202].

Biomodulators include a pharmacological group of drugs with natural or artificial origin, presumably able to increase non-specific resistance of the body to a wide range of harmful effects and stress.

The effect of flavonoids, namely curcumin, on cancer cells has been proven. Curcumin, a substance with a yellow pigment and a component of turmeric, significantly enhances mTOR (mechanistic target of rapamycin kinase) inhibition and induces apoptosis in kidney cancer cells [203].

A significant number of studies tested various feed additives, among which humic substances were used in livestock and poultry diets. Humic substances are usually present in nature because they are formed by the decomposition of organic matter and are usually found in soil and natural water. The active components of humic substances consist of humic acid, humus, ulmic acid, fulvic acid, humin, and some trace elements [204].

Humic acid has a nutritional property that improves neutrophil activity, which may protect against pathogenic bacteria and reduce mortality during acute bacterial infection. Finally, the effect of humic acid on enhancing immune functions may be conditioned by its antiviral properties, activation of neutrophils, and phagocytic activity of leukocytes [205].

Analyzing the data presented in our studies, it should be noted that the most pronounced changes after applying Humilid, a humic-based biologically active feed additive, were related to the content of total protein and γ-globulin fraction in blood serum [206].

Under the influence of Humilid in piglets, the content of lactic and pyruvic acids decreased in blood serum but α-amylase activity, the content of glucose, total protein, globulins, hemoglobin, and the number of erythrocytes increased. In animals that were prescribed Humilid, the average daily body weight gain was 10.8% higher compared with the control [207].

It is important that the inclusion of Humilid, a humic-based biologically active feed additive, in the main diet of domestic animals does not cause sensitization of the organism to its active ingredients. On the contrary, the inclusion of Humilid in the main diet of domestic animals has a desensitizing effect, which is evidenced by a decrease in the number of blood eosinophils in the experimental animals. The main quantitative changes in the population of blood cells under the influence of this biologically active compound are related to the cellular innate immunity, as evidenced by an increase in the total number of granulocytes and monocytes [206].

Despite the fact that dozens of natural and synthetic immunomodulators (Olexin, Rhidostin, Imunofan, Polyoxidonium, Neovir, Leukinferon, Cycloferon, Roncoleukin (recombinant IL-2), Betaleukin, Vilon, and Thymogen) are widely used in modern clinical and experimental practice, their application is limited for a number of reasons. For example, such limitations include the selective stimulating effect of a particular drug on one of the parts of the immune system, the lack of data on the mode of action in the body against the background of the infectious process, and quite high allergenicity and toxicity [208].

One of the correctors of secondary immunodeficiencies (including stress-induced), which activates stem hematopoietic cells and macrophages, has adjuvant and, unlike most other immunomodulators, pronounced anti-inflammatory activity, is the drug Fosprenil – a product of the phosphorylation of polyprenols of pine needles [209]. The first studies of Fosprenil began in the mid-80s of the last century. In a very brief research period, it was found that this compound has a pronounced immunomodulatory activity. Then antiviral activity of Fosprenil was proven both *in vitro* (regarding human immunodeficiency viruses, hepatitis A, and canine distemper virus) and *in vivo* (in the treatment of experimental infections caused by tick-borne encephalitis and ectromelia

viruses). For many years, scientists from various institutes could not identify viruses that are resistant to the drug [210].

The studied facts make it possible to assume that the mechanism of immunoregulatory ability of Fosprenil is as follows: endogenous phosphates of polyprenols participate in the biosynthesis of N-glycan chains of glycoproteins, which include, in particular, all types of immunoglobulins (Ig A, Ig G, and Ig M), g- and b-interferons, surface antigens, and almost all cell surface receptors. Probably that during secondary immunodeficiencies, there is a lack of endogenous polyprenol phosphates in the cells, so the introduction of Fosprenil into the body normalizes the physiology and functions of immunocompetent cells [210].

Research conducted in dogs showed that when Fosprenil was administered, the number of blood lymphocytes decreased by 3.7% ($p < 0.01$) in animals of the experimental group. On the other hand, this indicator didn't decrease in the control animals. In experimental animals, there was also a decrease in the number of T cells by 6% ($p < 0.001$) and this occurred only at the expense of cells with helper activity (by 5.36%; $p < 0.001$), and, on the contrary, the number of cells with cytotoxic (suppressor) activity had a slight tendency to increase (by 0.9%). Such changes in the relative number of T cell subpopulations testify to the immunoregulatory effect of the studied drug, which was expressed by an increase in the level of antiviral protection (increase in T suppressor cells). At the same time, T cell subpopulations in animals of the control group did not change significantly. We emphasize that the decrease in the proliferation of T cells is not related to the suppressive relationship of two major subtypes of T helper cells [211].

From the obtained data, it can be concluded that sodium polyprenyl phosphate directly affects the cellular immunity in a receptor-mediated way. It is assumed that it acts on the assembly or interaction of the viral particle with the receptors of the target cell, as a result of which the infection either does not develop, or defective particles are formed. Another mechanism of antiviral action of Fospenil may be related to its ability to increase the body's natural resistance. It was found that Fosprenil activates macrophages and causes the production of IFN and TNF-α [212,213].

Correction of disorders of the immune system using immunoactive drugs is a promising direction of research, and the search for such drugs, as well as methods of their use, is an urgent issue for animal husbandry, as stated by most authors [214, 215]. Interferon inducers are often used as correctors of immune status disorders, for example, in various immunodeficiency states, allergies, etc. Violation of immunological functions may be associated with a genetic or acquired defect. As is known, immunodeficiencies are disorders of immunological reactivity caused by the loss of one or more components of the immune system or non-specific factors closely interacting with it [212].

When studying the immunomodulating properties of Cycloferon, the authors found that the injection of this agent causes a decrease in the titer of specific immunoglobulins against canine distemper virus in blood of puppies, on average, by 1.18 U/mL, and against parvovirus enteritis – by 182.3 U/mL ($p < 0.01$) after 14 days. Thus, the titer of specific antibodies against both diseases significantly decreased. At the same time, an increase in the absolute and relative number of B cells was noted as a compensatory reaction in 14 days after the administration of the drug. The use of Cycloferon in humans had a slightly different effect. This led to a decrease in the level of B cells in peripheral blood and to an increase in the production of high-affinity antibodies, which may reflect the effect of this drug on switching the synthesis of immunoglobulin classes in B cells [196].

The specified secondary effects of Cycloferon can be partially explained by the induction of the synthesis of IL-2, IL-1, IFN-α, and IFN-γ interleukins by various cells of the immune and other systems in the body; as well as inhibiting the synthesis of IL-8 and TNF-α. In addition, based on the obtained data, it can be assumed that Cycloferon is capable of inducing the synthesis of IL-10 and/or TGF-β [216].

When analyzing the phagocytic activity of neutrophils, it was found that their activity significantly decreased by 65.83% ($p < 0.01$) in 14 days after the administration of the immunotropic agent. A decrease in the phagocytic activity of neutrophils may be associated with the activation of immunoregulatory mechanisms in the body of puppies and, as a result, the release of harmful metabolites into the blood due to the

normalization of metabolic processes.

At the same time, the administration of Cycloferon in animals increases the receptor activity of lymphocytes, which is manifested by an increase in the percentage of inversion to herpes antigen and interferon by 2.2 and 2.9 times, respectively.

After the administration of Cycloferon in puppies, there was an increase in the absolute number of lymphocytes by 34.99% ($p < 0.001$). At the same time, the absolute number of leukocytes had a tendency to decrease by 2.67% ($p < 0.01$). In the dynamics, a significant increase in the absolute number of T cells by 30.71% ($p < 0.01$) was also noted before and after the administration of the drug. The absolute number of T helper cells increased by 41.36% ($p < 0.001$) and the absolute number of T suppressor cells, on the contrary, significantly decreased by 10.61% ($p < 0.01$) after the administration of the drug.

Cycloferon has direct and indirect immunotropic properties. The administration of Cycloferon during secondary immunodeficiencies leads to significant changes in the composition of lymphocyte subpopulations: the relative and absolute number of initially reduced total T cells ($CD3^+$), T helper cells ($CD4^+$), NK cells, and immunoregulatory index increases [216].

The next author study was devoted to the determination of the effect of membrane plasmapheresis on indicators of cellular immunity. During the metabolism of all food products, especially proteins of animal origin, a large number of toxic products are formed in the body – various aldehydes, ketones, urea and its derivatives, and others [210, 213]. Any toxic substances must be immediately bound and evacuated. In case of violations of the body's release of toxic substances, intoxication occurs, which is associated with inadequate functioning of organs and systems.

One of the methods of detoxification in the human body is plasmapheresis, which affects the effective functioning of the immune system and is important for restoring its functioning [217].

According to research results, after the first procedure, the absolute number of lymphocytes in blood decreased by 3.08 g/L (by 26.29%; $p < 0.01$) in animals of the experimental group. In animals of the control group, a decrease by only 0.6 g/L (5.49%;

$p < 0.05$) was found. The second plasmapheresis procedure contributed to an increase in the absolute number of leukocytes to 9.4 ± 0.81 g/L ($p < 0.01$) in animals of the experimental group compared with the values after the first procedure. At the same time, the indicator remained unchanged in the control (10.4 ± 2.75 g/L). Before the plasmapheresis procedure, the absolute number of lymphocytes in the control and experimental groups did not differ significantly and were within physiological limits.

The most pronounced effect after the first plasmapheresis procedure was a significant decrease in the absolute number of phagocytizing neutrophils in dogs of the experimental group from 4.463 ± 0.164 before the procedure to 2.359 ± 0.180 g/L after it, i.e. by 47.14% ($p < 0.05$).

After a plasmapheresis session, a significant decrease in the concentration of pathological products can be observed in the blood but their content approaches the initial level after a few hours. This indicates that substances previously located in the interstitium or even in the cells entered the vascular bed [310]. The following plasmapheresis sessions contribute to the removal of these substances, which leads to a more complete sanitation of the entire internal environment, considering that the main part of harmful products are in the extravascular spaces. At the same time, it is necessary to take into account the fact that there is a “moving equilibrium” of concentrations of various substances in the intracellular, extracellular (interstitial), and intravascular spaces in the body [217]. A change in their content in one of these spaces (in this case – intravascular) leads to their redistribution in others. Thus, both xenobiotics, which are in the body for a long time or have enetred from the environment, and natural pathological metabolites can be removed from the body [217].

Therefore, the research performed by the authors demonstrates the dynamics of the changes in cellular immunity and the population composition of immunocompetent cells in dogs during plasmapheresis. The author’s data are consistent with data obtained during plasmapheresis in humans [217].

Many drugs have low therapeutic efficacy, that is, the concentration at which they exert a therapeutic effect differs little from the concentration at which the drug

becomes toxic. In other cases, drugs can rapidly lose their activity when administered in the body under the influence of inactivating agents. The inclusion of such drugs in liposomes can significantly increase their therapeutic efficacy, since, on the one hand, the drug contained in the liposome is protected by its membrane from adverse factors, and on the other hand, this membrane does not allow the toxic drug to exceed the permissible concentration in the body's biological fluids [218]. At the same time, the liposome acts as a carrier from which the drug is released gradually, in the required doses and places, and over a certain period of time.

In view of the above, the following author studies were aimed at studying the dynamics of indicators of immunophysiological status in dogs after the administration of Methisazone and Albuvir in the form of a liposomal emulsion. Already after the first administration of these biologically active substances, changes in immunogram indicators were noted, which were expressed by an increase in the number of leukocytes in both experimental groups; moreover, in animals that were administered Methisazone, they were more significant: 42% versus 12% ($p < 0.01$). The absolute number of phagocytic neutrophils significantly increased by 119.31% ($p < 0.01$) in animals treated with Methisazone after the first administration of the drug. In animals treated with Albuvir, this indicator also significantly increased by 15.58% ($p < 0.01$) after the administration of the drug.

The administration of biologically active substances with an immunotropic action resulted in a decrease in the phagocytic activity of neutrophils. This is accompanied by the activation of the cytotoxic effect of T suppressor cells and NK cells, which is probably a compensatory reaction to the active enter of metabolites into the general bloodstream and is regulated by signal cytokines in a receptor-mediated way [213]. Thus, the analysis of the state of the body's immunoreactivity after the administration of biologically active substances in the form of liposomal emulsions allows to assert that with certain changes in the immunoreactivity, in which proliferative cellular processes and a decrease in the activity of humoral factors and natural antiviral protection are detected, with the aim of correcting immunoregulatory mechanisms, is it is advisable to use biologically active substances in the form of

liposomal emulsions, in particular Albuvir and Methisazone.

Vitamins are extremely important for normal metabolism and vital activity of living organisms. Liposomal emulsions, in addition to structural components and main active ingredients of immunotropic action, also include fat-soluble vitamins A and E.

Assessment of vitamin E content in blood serum in animals treated with Albuvir and Methisazone showed a tendency to a substantial increase. At the same time, it should be noted that in animals treated with Albuvir, the increase was 4.98 μg/mL, and in animals treated with Methisazone – 4.01 μg/mL. After the first administration of the liposomal emulsion, there was a more pronounced increase in the content of this vitamin in blood serum than after the second. In animals treated with Albuvir during the experiment, namely after the two-time administration of liposomal emulsion, a more significant increase in the content of vitamin A was observed. Vitamin E can also inhibit various inflammatory processes by blocking the activity of NF-kB transcription factor, which is important for the transcription of many proteins, especially pro-inflammatory cytokines [219].

When analyzing the dynamics of the number of NK cells after the administration of liposomal emulsion, we observe somewhat similar results to the dynamics of T cells with suppressive activity in animals treated with Albuvir. That is, a significant antiproliferative effect was observed, which is expressed in a decrease in the number of these cells during the experiment. In animals treated with Methisazone, a single administration of the drug was accompanied by a sharp increase in the number of these cells, but a tendency to decrease was observed after repeated administration,.

On the basis of the conducted research, an active tactic for determining sensitization to antigens and immunomodulators in animals is proposed. If high sensitization to certain antigens is revealed, it is necessary to prescribe specific drugs with protective properties that will prevent manifestations of violations in the main regulatory systems and increase the efficiency of immunocorrective measures. When excessive autosensitization of the animal organism (more than 40%) to immunotropic agents is determined, their use is not allowed due to the possible manifestation of an immediate hypersensitivity reaction.

One of the factors that significantly affects the formation of adaptive immunity, especially during vaccination, are specific antibodies, the persistence of which is possible if the pathogen is present in the body. Given the significant spread of viral pathogens in urban areas and the absence of the control provided by the veterinary service over private dog kennels, the risk of persistence of viruses in the body of dogs at the time of vaccine administration is quite high. In order to avoid the immune system dysfunctions, the authors suggest to use the immunomodulators Trifuzol or Cycloferon before administering the vaccine, if it is not possible to determine the IgG titer.

It is an established fact that reproductive problems in dogs are often associated with immuno-endocrine disorders of the endometrium [220].

The average concentration of progesterone in all female dogs in our study begins to rise from 5 to 10 days, and the indicator of 6.48±0.54 nmol/l indicates that on the 10th day there was a surge of luteinizing hormone (LH), which is necessary for follicle ovulation. This finding is consistent with a large number of previous studies in which ovulation occurs approximately two days after the LH surge [221, 222] and is associated with a sharp increase in progesterone concentration [223], reflecting the increased level of luteinization associated with ovulation of many follicles.

The peak concentration of progesterone occurs on the 20th day of estrus. The reaction of the immune system, which is manifested by the maximum decrease in the phagocytic activity of neutrophils, should be noted. This fact points to the immunoregulatory function of progesterone, which obviously has physiological significance and consists in preparation for possible pregnancy. In the dog, implantation occurs around the 17th day of embryonic life, and the balance between pro- and anti-inflammatory reactions in fetal-maternal communication is crucial for the onset of pregnancy. Understanding of such immune mechanisms in canine reproduction is still insufficient [224].

An increase in the concentration of estradiol on the 5th day of estrus, in contrast to progesterone, had the opposite effect on PAN, namely, the ability of neutrophils to phagocytosis increased

Physiologically, increase of PAN and concentration of estradiol-17β on the 5th

day, in our opinion, is related to the need to rehabilitate the reproductive tract and prevent the possible development of inflammatory processes.

With an increase in the concentration of estradiol-17β on the 5th day, the content of lymphocytes decreases. This may indicate the suppressive effect of this hormone on adaptive immunity, namely on the most reactogenic cells of the body, in order to prevent autoaggression

In our study, the specific dynamics of cortisol during estrus was observed. This is expressed in the fact that during the follicular phase of the sexual cycle (up to 10th day), the concentration of this hormone increases, and on the 15th day, authors note its sharp decrease from 138.3±38.64 to 68.5±21.95 (2 times). Stress-like increases of glucocorticoids in plasma inhibit gonadotropin secretion and may disrupt ovarian cycling.

An increase in the concentration of T3 on the 10th day of the estrus was also noted and is probably associated with the provision of luteinization of postovulatory follicles

To prevent the development of stress-induced immunodeficiencies, especially in dogs that are in a state of chronic stress, the systematic use of drugs of natural origin that have adaptogenic properties is recommended. To ensure receptor unblocking and enhancing the functional activity of hematopoietic organs, phagocytic and immunocompetent cells, as well as providing adequate immunity correction, it is recommended to perform plasmapheresis procedures before prescribing immunomodulators. Appropriate actions will allow to reduce the antigenic load on immunocompetent cells and the number of circulating immune complexes, as well as improve the efficiency of the use of immunotropic agents.

Further studies of the immunomodulatory function of pregnancy-related hormones will improve our understanding of endocrine-immune interactions before and during pregnancy and may help to develop selective strategies for the treatment of infertility and pregnancy complications.

In case if systematic injection of immunomodulators is impossible and in the absence of their oral forms, an alternative can be liposomal emulsions as a carrier from

which the immunomodulator is gradually released. Besides the above, the addition of vitamins to such a dosage form contributes to the combined effect during immunity correction.

CONCLUSIONS AND PRACTICAL RECOMMENDATIONS

Biologically active substances with immunotropic action significantly affect different parts of cellular and humoral immunity. There is a certain degree of autosensitization of the organism to any immunologic agent, which can be determined by the percentage of inversion of activated T cells. This provides the opportunity to prevent possible manifestations of immediate hypersensitivity during the administration of immunologic agents and to optimize the efficiency of the implementation of immune corrective measures. The conducted experimental studies are the rationale for using the loading test of spontaneous rosette formation in the reaction with activated T cells in the case of the prescription of biologically active substances with immunotropic action during immunity correction in veterinary practice. In the case if inversion is 40% and above – it is concluded that there is hypersensitivity to the immunologic agent and thus its use is prohibited.

The comparative analysis of immunograms during the course of the administration of an immunologic agent, to which the optimal individual sensitivity in dogs is determined, shown that when immunologic agents are used in the recommended doses, the sensitivity of lymphocytes to these drugs decreases.

Inclusion of Humilid, a humic-based biologically active feed additive, in the main diet of domestic animals does not cause sensitization of the organism to the components of the active ingredients contained in the feed additive. This, in contrast, has a desensitizing effect, as evidenced by a decrease in the number of blood eosinophils in the experimental animals. The main quantitative changes in the population of blood cells due to the effect of this feed additive are related to the cellular

component of the innate immunity, as evidenced by the increase in the total number of granulocytes and monocytes.

Cycloferon reduces specific antibody titers in puppies within 14 days after the administration and against this background compensatory increases the absolute and relative number of B cells. The administration of this biologically active agent stimulates T helper cell activity and reduces the ability of neutrophils to phagocytosis.

Plasmapheresis procedures as a means of corrective action for the immune system are accompanied by a gradual increase in the number of leukocytes and lymphocytes, as well as their subpopulations, and a pronounced decrease in the ability of neutrophilic granulocytes to phagocytosis, as the procedures are carried out.

Based on the described experimental results, it can be confirmed that the use of Trifuzol in a form of liposomal emulsion reduces the specific (titer of IgG antibodies against CDV and CPV) and enhances the non-specific immune response. Given such changes in cellular and humoral immunity, it is recommended to use Trifuzol 7–14 days before vaccination to inactivate circulating IgG due to passive immunization.

In accordance with the criteria established in studies of the effect of immunologic agents in the form of liposomal emulsions on the immunophysiological status in dogs, it should be assumed that these biologically active substances in this dosage form cause changes in population of immunocompetent cells. This is expressed by the antiproliferative effect on T and B cells, an increase in the absolute number of leukocytes, in particular T suppressor cells and NK cells, and a decrease in phagocytic activity of neutrophils.

The effect of progesterone on PAN in the luteal phase of the sexual cycle proves that immunosuppression is obviously physiologically justified in this period.

Therefore, in order to correct the immune system in animals, the following therapeutic methods are recommended:

– before prescribing an immunologic agent, determine the individual sensitivity of the organism to it. As a technique, it is possible to use a loading test with activated T cells;

– when prescribing immunologic agents that interact with surface lymphocyte receptors, reduce the dose specified by the manufacturer in 3–5 times but increase the period of their administration to one month, followed by repeats at least three times a year;

– in case of chronic diseases in dogs before prescribing an immunologic agent, it is necessary to perform three plasmapheresis procedures;

– in case of vaccine administration, especially in the postnatal period, it is necessary to preliminarily conduct a course of immunity correction using drugs Cycloferon 10 days before vaccination or Trifuzol 5 days before vaccination;

– in order to increase the adaptive capacity of the immune system, it is necessary to systematically use adaptogens in the canine diet. One of such means is Humilid – a humic-based biologically active feed additive.

RESUME

In the modern conditions, immunological blood tests become extremely relevant in clinical practice. By using the results of these tests it is possible to quantify the indicators of non-specific resistance, cellular and humoral immunity, and when studying them in dynamics – to asses the efficiency of treatment courses and develop methods to correct immunodeficiencies. Studies conducted on small domestic animals in large cities have revealed significant changes in immunological status, which leaves an imprint on the course of infectious and non-infectious pathology. The pharmaceutical industry offers a wide range of immunomodulators that affect various parts of innate and adaptive immunity in animals. The key in clinical practice is to determine the individual sensitivity of the animal organism to an immunotropic agent and the prescription of a scheme for immunity correction. It should be noted that violations in the immune system may begin long before the clinical manifestation and manifest themselves under the exposure to a spontaneous biological stimulus. The above indicates the necessity to take into account the formation of adaptive immunity in dogs immediately after birth and, especially in critical periods of the development, emphasizing attention on the period of vaccine administration. The presented results of the author studies allow to gain knowledge about the ambiguity of the mechanisms of regulatory effect of immunoregulators with different origin and plasmapheresis on the functioning of T and B cells in adaptive immunity.

The results of the research presented in this monograph will not only complement the basic knowledge, but will also help veterinarians to better understand the immune system and how it is affected by various agents.

The monograph will be useful to scientists and doctors, as well as to applicants for higher education in the field of veterinary medicine.

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NOTES

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