Approaches to the study of functional activity phagocytic link immune system (review)

**Abstract:** Literature data on the role and significance of phagocytic component, in particular, neutrophils in realization of immune response of an organism to antigen are analyzed. A special attention is paid to approaches to evaluation of functional state and reactivity of phagocytes. The most informative and objective method for evaluation of functional state of neutrophils is NBT-test. NBT-test allows to evaluate a potential maximal ability of neutrophils (stimulation test), what part of maximal ability neutrophils realize at the moment of examination (spontaneous test), degree of additional activation of neutrophils in response to antigen stimulation. Spectrophotometric modification of NBT-test permits to carry out many determinations simultaneously.

**Keywords:** phagocytic component of immune system, nitro blue tetrazolium reduction by neutrophils test (NBT-test).

In recent years, due to the deterioration of the environment and social conditions of the population increases the impact of negative factors on human health and the adaptive capacity of the body, including the immune system. There is a decrease of functional activity of mononuclear phagocyte system cells, T and B-lymphocytes, in particular, the development of post-vaccination immunity is broken and as a result, reduces the effectiveness of preventive vaccination and herd immunity level [32]. Differential response of phagocytic cells level, depending on the biological properties of bacterial infectious agents, plays a leading role at the stage of initiation of infectious processes [14; 15].

To date, determined the position of a single phagocytic immune system provides the body's resistance to infectious pathogens [14, 36]. The name of the system implies that along with lymphocytic cells in the formation of cellular and humoral immune responses participate mononuclear and polyphagocytes, which in turn relate to the central unit of nonspecific protection [28]. It is difficult to find such a change in the internal environment of the organism, which is not recorded phagocytosis system. As powerful effectors, phagocytes are transformed into a communication center, a kind of strategic target, by which transformed all the reactions of the blood and connective tissue. With the penetration of the microbe in the first macro-organism cell that comes into the fight with him, it is a tissue macrophage. It absorbs and digests microbe's antigenic peptides representing T and B cells and thereby initiating the development of cellular and humoral response. Thus, macrophage allocates cytokines — interleukins 1, 6, 8, 12, and FNO that activate nonspecific resistance factors: neutrophils, monocytes, NK-cells — and act on T and B-lymphocytes, promoting the development of specific immunity. Thus, macrophages and other antigen-presenting cells are the first cells that initiate the development of non-specific resistance and specific immunity [26].

In addition to macrophages, neutrophils play an important role in the realization of the innate immune mechanisms. These cells predominate in the early cellular infiltrate at the site of inflammation. They are in the blood and virtually absent in normal tissues. Neutrophils — are short-lived cells. On average, their life is 3–4 days. By sharing in circulation every 5 hours, they are «photographed» changes that occur during this period, as a kind of mirror of homeostasis. With the implementation of the innate immune response produced by a variety of factors, and they quickly emigrate from the blood and enter the site of infection. The focus of neutrophils are able to eliminate many pathogens by phagocytosis [5; 6; 18].

In recent years, it found that neutrophils are closely interact with virtually all the humoral (immunoglobulins, complement, coagulation, kallikrein system, fibrinolysis system) and cellular (macrophages, lymphocytes, platelets, basophils, eosinophils, endothelial cells, fibroblasts), blood and connective tissues system involved in maintaining homeostasis [7; 17]. The cooperative processes neutrophil, on the one hand, acts as a target cell, and on the other — as a cell regulator. All regulatory neutrophil reaction is carried out using a variety of mediators secreted from the cell into the pericellular environment: leukotrienes, prostaglandins, enzymes, cytokines — neutrophilokine, metabolites respiratory burst [13].

According to modern concepts, there are several mechanisms for cooperation of neutrophils and macrophages, the first — by neutrophils neutrophilokine able to modify the functional activity of macrophages; the second — neutrophils stimulate the bactericidal activity of macrophages by resorption latest neutrophil degradation products; third — neutrophils partially or completely neutralize secreted by the bacteria into the extracellular space agents [27].

It is known that phagocytosis process has the sharp increase in the consumption of oxygen by phagocytic cells (neutrophils, monocytes) of peripheral blood. This phenomenon, figuratively name «respiratory, metabolic or respiratory burst», it is one of the effector mechanisms (possibly leading) bactericidal cells. The essence of this phenomenon is reduced to a pronounced activation of membrane

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oxidases that catalyze electron transfer from NADPH to molecular oxygen oxidation of glucose in the hexose monophosphate shunt, overproduction of reactive oxygen species and oxygen peroxide. These active forms of oxygen and hydrogen peroxide are biological oxidants with high cytotoxic activity, causes the final result of phagocytosis — killing microbes [19; 30].

One of the promising areas of modern experimental Immunology is the study of the influence of various endogenous and exogenous peptides as well as their synthetic analogues on the functioning of immune cells, including neutrophils [6; 7; 37; 38]. Functional sensing neutrophils, which are the first line of defense of the body and determine the development of a specific immune response, to evaluate not only their effector resources and reserves immunity [2].

The results indicate that the level of activity of the cytochemical changes of intracellular components of neutrophil system reflects the depth and severity of the disease process and the status of non-specific reactivity in patients with various forms of diseases of infectious and non-infectious origin [3; 20; 22; 25; 34].

In our study, the task to study the relationship of humoral, cellular and innate immunity in response to antigens typhoid bacteria through vaccination, as well to develop a sustainable approach in the diagnosis of disorders of the immune response both in vivo, and in vitro using modern tests. One of the important trends in the development of modern immunology and infectious disease is the desire of experts to consider a broad and diverse set of biological factors related to the main categories of specific and nonspecific immunity, as a single functional complex. This agrees well with the data showing the importance of non-specific metabolic stimulation of lymphocytes for a specific phase of the immune response [4; 10].

It is no coincidence that the indicator tests based on high poly-nuclears reactivity has been used in the clinic for a long time and on the information content is often superior to other hematologic parameters. Of the many methods proposed in recent years, stands out assessment of reactive changes in the system of oxidative (oxygen-dependent) metabolism, which is largely due implementation of the effector features of phagocytosis [8; 17; 21]. Cytochemical methods for studying leukocytes are now widely used in medical practice for the study of the functioning and the degree of activation of phagocytic immunity in the dynamics of infectious process [19; 20; 21; 23].

Test recovery nitro blue tetrazolium (NBT-test) — the most widely used to quantify the degree of activity of intracellular enzyme systems phagocytic human and animal cells [4; 10]. Many studies show the undoubted role of NBT-test indices, its connection with other types of diagnostics and clinic different nature of infectious and somatic diseases [20]. NBT-test significantly earlier than other research methods to predict adverse outcome not only diseases but also for immunization, which makes it possible to apply effective treatment measures or immune corrective therapy to achieve adequate immune response to vaccination [24; 33]. For example, in typhoid fever with the outcome of the recovery, the dynamics of the NBT test performance indicates the maximum increase in metabolic and phagocytic activity of neutrophils in the blood during the height of the clinical manifestations and decreased activity in the period of early convalescence disease. From the early convalescence period to late convalescence, the indicators tend to further decline, but slightly higher than with those of healthy individuals. For the acute course of Salmonella infection is characterized by a high degree of frequency and oscillation parameters of cellular immunity and FMAN. Maximum implementation of immune mechanisms observed in the early stages of infection, in the later stages is characterized by stable dynamics of changes in the direction of their normalization [4; 9].

According Shukurova D. T. dynamics FMAN values in NBT-test has specific characteristics depending on the period and the disease. When acute course of gastrointestinal form of salmonellosis in children is a sharp activation of neutrophils in 2.8 times higher than normal values in the midst of their disease and a significant reduction in the period of convalescence. Such dynamics FMAN is characteristic of infection with acute course, followed by the complete elimination of the pathogen and the favorable outcome of the disease. On the other hand, if protracted course of the degree of activation of neutrophils moderate during the height of the disease and during convalescence neutrophil activity continues to rise. In other words, this dynamic is evaluated as an indicator of a weak immune response and high-risk chronic process [34].

An analysis of the literature data, epidemiological and clinical practice suggest the protective role of antitoxins in the occurrence of diphtheria. Healthy persons vaccinated during the year prior to the survey have normal levels of both cellular and humoral immunity and nonspecific factors of protection with a high level of sensitization of lymphocytes to diphtheria toxoid. Comparison of lymphocyte stimulation index of diphtheria toxoid in the vaccine group and without a history of vaccination of healthy persons makes it possible to use this reaction to assess the effectiveness of vaccination [1].

Value NBT-test is that it allows revealing the presence of «metabolic explosion» that arises in connection with neutrophils in the process of phagocytosis. We know that the implementation process of phagocytosis by neutrophils is accompanied by a sharp increase in oxygen consumption, the intensification of the hexose monophosphate shunt and the formation of hydrogen peroxide in these cells. The principle of the method is that NBT is a redox indicator, which reacts on phagocytic cells, subjected to reduction to insoluble Formosan under the influence of NADN oxidases free nucleotides and NADF [17; 29; 33].

The process of restoring by neutrophils colorless dye nitro blue tetrazolium to insoluble Formosan dark blue consists of two interconnected stages. The first stage — HCT own phagocytosis, which depends on the shell and granulocytes increases when it is damaged by the microorganism and its products or stimulating factors contact with the cell surface. The second stage — under the influence of oxidizing NADPH oxidase activity, an enzyme localized in lysosomes of granulocytes NBT to Formosan restored. It is believed that this process correlates with the intracellular formation of hydrogen peroxide, which, according to modern concepts, together with iodine forms an important bactericidal neutrophil granulocytes system. Stimulation of neutrophils dis-inhibits NADF, diaphorase and activates the hexose monophosphate shunt. The electrons are taken from the NADF, convert molecular oxygen to superoxide anion, which owns a direct role in restoring the NST. In general, NBT-test reflects the degree of activation of the oxygen metabolism, the function of the hexose monophosphate shunt and related free radicals operating time [19].

However, a significant drawback cytochemical studies are the high cost of time for consideration of the results in blood smears reaction. One way to reduce the time and objectivity of the results is a recovery test neutrophils nitro blue tetrazolium (NBT-test), which is conducted quantitative spectrophotometric method. Modification of this method enables to determine FMAN in large numbers of samples, a significant reduction of time and the possibility of counting results of research as a digestive ability of neutrophils in vivo, or in vitro. The advantages of the developed method is the ability to obtain information about the intracellular metabolism of neutrophils objective spectrophotometric method using a standard ELISA plate and enzyme immunoassay analyzer [8; 11; 12; 35; 36].
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Dynamics of clinical and immunological parameters of pharmacotherapy of pulmonary hypertension in patients with CHD in the surgical treatment stages

Abstract: Patients with congenital heart disease complicated by pulmonary hypertension in preoperative treatment were administered phosphodiesterase-5 inhibitors (iPDE-5) and ACE inhibitors (ACEI)-captopril, which promote vasodilation of the pulmonary circulation, reduce lung resistance and the pressure in the pulmonary artery by 10–14%. The use of combined therapy in the postoperative period, including inotropic agents- dopamine, dobutamin, epinephrine; phosphodiesterase-3 inhibitors (iPDE-3) — milremone, enoximone, perfan; iPDE-5, nitrates; prostaglandins E1-vasoprostan, which reduce the development of severe cardiovascular failure in the short term, prevent pulmonary hypertension crises and further reduce residual pulmonary hypertension by another 8–10%.

Thus, drugs of iPDE-5 can be recommended as the first line drug for the treatment of PH in the preoperative period and as a maintenance therapy in patients with inoperable IIIb-IV level of PH, improving the quality and extending the life span. Combined therapy with these drugs can be recommended in early postoperative period, as an effective therapy aimed at preventing pulmonary hypertensive crisis, treatment of cardiovascular failure, the further reduction of residual PH, and thus, improvement of patients’ condition in the late postoperative period.

On 52 patients between the ages of 3 to 14 with congenital heart failure, complicated pulmonary hypertension on 3rd degree are learned dynamic of cellular immunity on surgical treatment depend on different pharmacotherapy. Treatment was consist of traditional therapy in patient who have congenital heart failure with pulmonary hypertension inhibitors of phosphodiesterase-5 (iPDE-5) (Viagra, Pfizer) and inhibitors AFP (iAFP) shows with time length increasing tendency their favorable impact on character and degree of severity in patient with secondary immunodeficiency.

Keywords: congenital heart disease, pulmonary hypertension, cellular immunity, secondary immunodeficiency, pulmonary hypertensive circulation, inhibitors phosphodiesterase-5 (iPDE).