PHAGOCYTIC ACTIVITY OF NEUTROPHILS AS A MARKER OF BACTERIAL, VIRAL AND FUNGAL INFLAMMATION IN MALE PROSTATE

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Introduction. Phagocytosis at first noted by Canadian physician William Osler in 1876 and then studied and described by Dr. Ilya Mechnikov in 1882, when he established the role of phagocytes for bacterial infection. In those time 1870-1882 Mechnikov worked in our Odesa University. The discovery of phagocytosis was made by Dr. Ilya Mechnikov, a pioneering scientist in the fields of immunology. The term phagocytosis derives from the Greek words phagein and kytos, which roughly translates to the phrase (to devour cell)- fittingly describing what this process appears to resemble under the microscope.

In few words phagocytosis (neutrophil phagocityc activity) is the process of phagocyte distribution of microbial cells, viruses or natural cells of the body. The study of phagocytosis allows us to identify a infringement of the cellular link of immunity. Phagocytes it is cells which protect body by absorbcion harmful heterogenous particles: bacteria, viruses, fungus and dead or dying cells. Phagocytosis is a central element of the microbicidal function of neutrophils. Due to testing of phagocitic activity neutrophis this analisys allows to assesst condition of immune system. Phagocytosis it is ability of white blood cells more exactly to neutrophils and monocytes to swallow, devour and digest heterogenous proteins such as bacteria, viruses, fungus. After complete destruction of pathogenic microorganisms the phagocyte itself is destroyed.

Phagocytosis is a process wherein a cell binds to the item it wants to engulf on the cell surface and draws the item inward while engulfing around it. The process of phagocytosis often happens when the cell is trying to destroy something, like a virus, bacteria or fungus an infected cell, and is often used by immune system cells.

Phagocytosis are the 6 stages: step 1. Chemotaxis: phagocyte is attracted or called towards infection. 2. Adherence: phagocyte attaches to microbe. 3. Ingestion: microbe is engulfed in "phagosome". 4. Phagolysosome formation: lysosome adds digestive chemicals. 5. Killing. 6. Elimination.

Phagocytosis is the mechanism by which relatively large (>0.5 μm) particles, such as bacteria, viruses, fungus, dead cells, or polystyrene beads, are internalized.

There are many classes of phagocytes within the body, each with different specialized functions involving phagocytosis. Most phagocytes are derived from stem cells in the bone marrow. The main types of phagocytes are monocytes, macrophages, neutrophils, tissue dendritic cells, and mast cells. A type of immune cell that can surround and kill microorganisms, ingest foreign material, and remove dead cells. It can also boost immune responses. Monocytes, macrophages, and neutrophils are phagocytes.

Macrophages are specialised cells involved in the detection, phagocytosis and destruction of bacteria and other harmful organisms. In addition, they can also present

antigens to T-cells and initiate inflammation by releasing molecules (known as cytokines) that activate other cells.

Natural killer cells (NK cells) are white blood cells that destroy infected and diseased cells, like cancer cells. They are also a type of lymphocyte, like B-cells and T-cells. When microorganisms, such as bacteria or viruses, enter the body, neutrophils are one of the first immune cells to respond. They travel to the site of infection, where they destroy the microorganisms by ingesting them and releasing enzymes that kill them. Neutrophils also boost the response of other immune cells.

The phagosome is the organelle formed by phagocytosis of material. It then moves toward the centrosome of the phagocyte and is fused with lysosomes, forming a phagolysosome and leading to degradation. Progressively, the phagolysosome is acidified, activating degradative enzymes.

Phagocytosis is a process that demands a large supply of energy, most likely to combat infections and remove debris in damaged tissues. Normally, glucose is the favored fuel to generate ATP.

Phagocytic cells also contain other receptors, such as scavenger receptors and LPS receptors, that stimulate phagocytosis without the use of an opsonin. Such receptors bind to ligands such as PAMPs and damage-associated molecular patterns (DAMPs) to stimulate phagocytosis.

Once inside this phagocyte, the bacterium is trapped in a compartment called a phagosome. Within one minute the phagosome merges with either a lysosome or a granule to form a phagolysosome. The bacterium is then subjected to an overwhelming array of killing mechanisms and is dead a few minutes later.

Phagocytosis is triggered when specific receptors on the phagocyte bind ligands on the microbe surface. Importantly, the receptors engaged during phagocytosis and subsequent signaling events modulate induction of the respiratory burst, phagosome-lysosome fusion, and consequently, the fate of the ingested microorganism.

Phagocytosis is a receptor-mediated process designed to engulf and destroy large target cells, including microbes, from the body. Professional phagocytes possess specialized receptors that can engage with the target directly or via intermediary products including opsonins.

Aim. To determine and to assesst functional possibility of neutrophils for immune protection of body against bacteria, viruses, and fungal pathogens for prostatitis sufferers. To establish the functional capacity of neutrophils in the body of chronic prostatitis sufferers with complete or incomplete phagocytosis.

Material and methods. For testing of phagocytosis usually used micromethod of blood. Amount of capillary blood samples - 1.0 ml. In test reaction add 0.1 ml heparin with gradient of density - 1.19 and add 0.1 ml physiological solution of sodium chloride, add 0.1 ml blood sample from finger. Test tube or tested plate necessary to centrifuge at 2000g. (Rcf) during 15 minute. Then twice wash off during 15 minutes, supernatant trow out and leave neutrophils in amount 2-4 x 10^6 cells. In reaction of testing necessary to take 0.05 ml of suspension of neutrophils and 0.05 ml suspension of boiled yeast culture *Saccharomyces cerevisiae*. Incubate tested plate at 37ºC during 30 minutes. Just put a smear of neutrophils on glass and dry it. After that the smear is stained with hematoxylin. Before to start test of phagocytosis necessary to prepare

yeast culture *Saccharomyces cerevisie*. In glass Erlenmeyer flask used for a variety of laboratory liquid handling applications add purified water 100.0 ml and 3.0g. of dried yeast *Saccharomyces cerevisiae* in 100.0 ml purified water. Thoroughly dissolve yeast and boil it in water bath during one hour. After one hour of boiling then cool the solution and resuspend, then centrifuge solution at rotation speed 3000g. during 15 minutes. Supernatant remove and use those sediment resuspend 2-3 times. Add the boiled sediment of particles *Saccharomyces cerevisiae* into the test tube and add 3.0 ml sterile purified water. Keep it in refrigerator at +4-6°C during 2 month. Dose yeast for use in blood test 0.1 ml dissolve with 10.0 ml purified water and take for phagocytosis testing.

Twenty-six men with confirmed chronic prostatitis in reproductive age (18-40 years old) were included in the research with their consent. Staining of phagicytic activity of neutrophils used by method of hemotoxylin-eosin.

To detect neutophil activity of phagocytes we did microscopy of blood cells. Microscope used model: NK-210T 40x-2500x Trinocular Biological Compound Microscope with bottom LED illumination (Ningbo Amz Instruments Co., Ltd. China). Microscope used for count of neutrophils in chronic prostatitis sufferes blood.

Testing and microscopy of phagocytic activity of neutrophils were done in the laboratory of immunology Filatov's Research Institute of Eye Disease and Tissue Therapy, Odesa, Ukraine.

To confirm chronic bacterial inflammation in prostate we used liquid media and agaric media to isolate *Staphylococcus aureus* and *Enterococcus faecalis*. (Jinan Baibo Biotechnology Co., Ltd., Shandong, China).

Results and discussion. We have an interest to incomplete phagocytosis in chronic prostatitis sufferers. During a chronic inflammation phagocytic activity of neutrophils decreasing and during pinocytosis neutrophil accepting microbial cell, but can not kill it and digest, therefore in opposite microbe kill neutrophil. The body therefore has chronic condition of not-complete phagocytosis and working useless until correcting work of phagocytosis up to complete process. Only therefore inflammation process continue with years and decades. Incomplete phagocytosis is the inability of phagocytes (mainly macrophages) to destroy internalized pathogens when these pathogens persist and can spread throughout the body. Phagocytosis is a cellular process for ingesting and eliminating particles larger than 0.5 μm in diameter, including microorganisms, foreign substances, and apoptotic cells.

Immunological deficiency considered in the pathogenesis of chronic prostatitis, despite clinical symptoms of prostatic inflammation. Investigations in twenty-six men chronic prostatitis sufferers with severe bacterial prostatitis with Gram-positive bacteria: *Staphylococcus aureus* and *Enterococcus faecalis* in the prostatic secretion revealed a decreased phagocytosis activity of the polymorphonuclear leukocytes (granulocyte, PMN-cells) derived from sufferers sera towards the autologous Gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis* from their own prostatic secretions. These observations indicate a hitherto unobserved, altered host-parasite interaction in chronic prostatitis sufferers caused by Gram-positive bacteria. The most common cases are when chronic prostatitis sufferers have incomplete phagocytosis. This is the reason for the long-term chronic inflammatory process in the

prostate continue. Phagocytes are not able to kill and digest microbial cells. Therefore, microbes kill neutrophils without feeling an obstacle. As soon as the phagocytic activity of neutrophils is restored and will be completed, neutrophils will absorb the microbial cell, kill it and digest it. Only in this case chronic inflammatory process in the prostate will be stopped and resolved.

Normal range phagocytic activity of neutrophils in men relative value 40-80% or absolute value 1600-4000 cells/L.

Bacterial and neutrophil counts were digitized to characterize the kinetics of neutrophil-mediated bacterial killing and inform on the immune systems contribution to the clearance of bacterial infections. In vitro dynamics of bacteria and the kinetics of neutrophil-mediated phagocytosis and digestion which was extended to in vivo studies in immune-competent and immune-compromised mice. Neutrophil-mediated bacterial killing was described by two first-order processes-phagocytosis and digestion-scaled by neutrophil concentration 50% of the maximum was achieved at neutrophil counts. The process efficiencies diminished as the phagocytosed bacteria to total neutrophils ratio increased (with 50% reduction at a ratio of 3.4). Predictions showed how the therapeutically induced reduction of neutrophil counts enabled bacterial growth, especially when falling below neutrophil cells, whereas control individuals could deal with all tested bacterial burdens. The model-based characterization of neutrophil-mediated bacterial killing simultaneously predicted data across in vitro and in vivo studies and may be used to inform the capacity of host-response at the individual level.

Conclusions. During research recognised that chronic inflammation in prostate connected with incomplete phagocytic activity of neutrophils. Gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis* were isolated from expressed prostatic secretion. Since phagocytic activity of neutrophils will be normalized and start to work completed, neutrophils will be accept microbial cells, kill them and digest them. Only in this case phagocitic activity will be recovered and body will start to work properly. This condition will remove chronic inflammation of prostate in sufferers. We noticed increasing phagocytic activity of neutrophils during chronic prostatitis inflammation where reason of inflammation bacteria *Staphylococcus aureus* and *Enterococcus faecalis.* This agrees the organism of body constantly fights chronic infection, however it is useless. Because functional phagocitic activity of neutrophils is incomplete. Pinocytosis is normal, and neutrophils swallows bacteria but not able to kill it and to digest, this is big problem for the chronic prostatitis sufferers body.

Keywords: phagocitic activity, neutrophil, macrophage, incomplete phagocytosis, microscopy.