MECHANISM OF IMMUNE PROTECTION TO ESCHERICHIA COLI AND ENTEROCOCCUS FAECALIS IN THE CHRONIC PROSTATITIS SUFFERERS

Bayraktar V. M.
Mechnikov National University, Odesa, Ukraine
virolviro6@gmail.com

Introduction: *Escherichia coli* is most frequent bacterial opportunistic infection, causing urethritis, cystitis, prostatitis and pyelonephritis. The Gram-negative bacteria stain red with fuchsin. They are facultative anaerobes, do not form endospores and are rod-shaped. As Gram-negative bacteria, they measure 0.4-0.8 x 1.0-3.0 μm, with a cell volume of 0.6-0.7 μm. The optimal temperature for grwoth is 37ºC, having flagella and able capable of mobility. *Escherichia coli* ferments lactose with gas producing, though both lactose positive and lactose-negative strains exists. They do not form spores and capseles. On Endo agar *Escherichia coli* typically shows metallic shine, thought some strains do not exhibit this characteristic.

Enterococcus faecalis is also a frequent bacterial opportunistic infection. *Enterococcus faecalis* cause of inflammatory processes in the urethral tract including urinary tract infections, urethritis, cystitis, prostatitis and vesiculitis.

Enterococcus faecalis is Gram-positive coccus that is non-motile and facultatively anaerobic. It does not produce spores but produces exotoxins. On blood agar, it shows gamma hemolysis. It can grow in high concentration of sodium chloride. It ferments without gas producing and is positive to catalase reaction with hydrogen peroxide. It catabolizes various energy sources including glycerol, lactate, malate, citrate, agrinine, agmatine, and many keto acids. It grows at temperatures from 10ºC up to 45ºC and survives at 60ºC for 30 minutes. *Enterococcus faecalis* has an oval form and size 0.6-2 μm to 0.6-2.5 μm.

Material and Methods: To isolate *Escherichia coli* and *Enterococcus faecalis* in samples of expressed prostatic fluid and sperm from prostatitis sufferes, we used liquid and dense agar media. Lactose Peptone Broth was used to accumulate and directly cultivate coliform bacteria. The media contains phenol red or bromothymol blue. A positive result for coliform bacteria shows yellow color of growth after 24 hours of cultivation. In thioglycollate medium tall column test tubes, growth appears as dots or inclusions visible throughout the column of media. To isolate separate bacterial colonies of *Escherichia coli* and *Enterococcus faecalis*, we inoculated samples in Endo Agar, which contains selective indicator 2,3,5-triphenyltetrazolium chloride making the color of bacterial colonies *Enterococcus faecalis* cherry red after 24 hours of cultivation at 37±0.2ºC.

Twenty-four men with chronic prostatitis in reproductive age (18-40 years old) were included in the research with their consent.

Lactose Peptone Broth, Thioglycollate medium, Enterococcosel Agar and Endo Agar obtained from Becton Dickinson Company, Maryland, USA.

For autoclaving media and other materials, we used a regime of 1.0 atm. pressure of heat steam, with a sterilization pressure of 1.0 millipascale (MPa) (1 kg/cm^2). We used a Steam Sterilizer GK-10-1 produced in a factory of medical equipment and

instruments, Tymen. Immune tests of blood were conducted in the laboratory of immunology at the Filatov Research Institute of Eye Desease and Tissue Therapy.

Results and Discussion: For specific protection against *Escherichia coli* and *Enterococcus faecalis* in chronic prostatitis sufferes, we used bacterial conjugate. For desensetization of the body against *Escherichia coli* and *Enterococcus faecais*, we used stepwise introduction of antigens. Before and after treatment, we monitored blood conditions of sufferers, including cellular and humoral immune system. For cellular immunity, we tested blood levels of: T-lymphocytes (CD3), T-helpers (CD4), T-supressors (CD8), natural killers (CD16), B-lymphocytes (CD19), and phagocitic activity of neutrophils (Ph); results are represented in table 1. For humoral immunity, we tested blood levels of immunoglobuline A, G and M results represented in table 2.

Cell link of immune status in blood M±m, n=24

o	Cellular immune	Normal range, %	Before treatment, %	After treatment, %
1.	Lymphocytes	19-37 %	36±4,1	33±3.18
2.	Neutrophils	47-72 %	64±5.82	57±4.30
3.	T-lymphocytes (CD3)	55-70 %	61±4.85	69±5.19
4.	T-helpers (CD4)	40-60 %	48±4.72	56±4.47
5.	T-supressors (CD8	10-20 %	14±1.37	16±1.63
6.	Natural killers (CD16)	10-20 %	21±2.16	14±1.48
7.	B-lymphocytes (CD19)	6-15 %	16±1.84	12±1.27
8.	Phagocitic activity of neutrophils (Ph)	40-80 %	82±6.39	62±4.31

Table 1 shows blood activity of prostatitis sufferes in baseline condition, where T-helpers, phagocytic activity of neutrophils, and natural killers were increased.

After the course of desensitization, one injection of bacterial conjugate prepared from *Escherichia coli* and *Enterococcus faecalis* was administered every third day for a total of 10 injections, with a gradual increase in dose. One course of desensetization consisted of 10 injections with three-day rest periods between injections, and the total time for desensitization took 37 days. Immune blood tests were necessary to repeat 1-1.5 month after the lat injection of conjugate to check what changes occurred during desensitization. As we can see from table 1, T-lymphocytes (CD_3) increased, T-helpers (CD_4) count also increased, while natural killers (CD_{16}), decreased and stabilized to normal levels. Phagocitic activity of neutrophils (Ph) also stabilized to normal amounts.

Specific protective immunization as a desensitization against *Escherichia coli* and *Enterococcus faecalis* gives the body of sufferers with chronic prostatitis the ability to increase specific protective antibodies after desensitization is completed. At the same time, it decreases body hypersensitivity to *Escherichia coli* and *Enterococcus faecalis* for further active immunization, but not earlier then two month after desensitization.

Humoral link of immune status in blood M±m, n=24

	Cellular immune	Normal range	Before treatment	After treatment
1.	Immunoglobuline A, g/L	1.2-2.0	2.81±0.63	2.46±0.51
2.	Immunoglobuline G, g/L	9.0-18.0	11.82±3.47	14.53±3.26
3.	Immunoglobuline M, g/L	0.9-1.2	1.64±0.39	0.87± 0.12

In the mechanism of pathophisiology of prostate inflammation, local immunity around the prostate plays a crucial role. If local immunity is low around the prostate, bacteria can easily penetrate prostatic tissue and initiate inflammation. If phagocytic activity is low phagocytes swallow bacteria but are unable to kill them. This incomplete phagocytosis allows the inflammatory process in the prostate to continue. Regarding humoral immunity, immunoglobulin A quickly react, and its concentration increases. The general task of immoglobulins in the body is to provide the first line of protection for mucosals, hindering virus and bacteria penetration into the body. Immunoglobuline A (AgA) does not have bactericidal properties; however, it participates in neutralization bacterial toxins. In the blood, IgA interacts with Fc receptors expresed by effector immune cells, resulting in inflammatory processes. This triggers phagocytic activity of monocytes, macrophages and neutrophils. Immunoglobuline M (IgM) forms first among antibodies when antigene penetrate the body. IgM is synthesized by plasma cells in the spleen and is most active in antibacterial immunity and other autoimmune diseases. Immunoglobulin G (IgG) it is general type of blood antibody and participates in controlling infections throughout the body, binding to different pathogens including viruses, bacteria, and fungi. The connection of IgG with pathogens leds to their immobilization and binding with one enother, known as agglutination. This allows phagocytes to recognize, ingulf, and kill pathogens. In discussion, during chronic inflammation in the prostate, incomplete phagocytosis exists, meaning phagocytes ingulf *Escherichia coli* and *Enterococcus faecalis* but are unable to digest the bacteria. Therefore, unable to kill the bacteria, they remain alive and continue to cause harm to the body. Once immunity is normalized and phagocytic activirty recovers, phagocytes will swollow bacteria and successfully digest and kill them. After that, phagocytosis will be complete, and the inflammatory process in the prostate will disappear. This is why it is necessary to and inflammatory problem in prostate will disappeare. What is why necessary to strart desensitization in

prostatitis sufferers and then, after time, begin active immunization to specifically protect the body.

Conclusions: *Escherichia coli* and *Enterococcus faecalis* were isolated from chronic prostatitis sufferers using classical microbiological methods.

Immune blood tests were conducted before treatment and 1.5 month after the last injection of immunoconjugate following desensetization.

Desensetization of sufferes bodies was confirmed to normalize phagicytic activity of neutrophils, T-heplers, and natural killers. Immunoglobulin A concentration decreased to normal range.

Keywords: chronic prostatitis, *Escherichia coli*, *Enterococcus faecalis*, Endo Agar, Enterococcosel Agar, bacterial conjugate, desensetization.