DESENSITIZATION OF PROSTATITIS SUFFERERS USING IMMUNOGEN-CONJUGATED CHLAMYDOPHILA PSITTACI VACCINE

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

Introduction. In cases when prostatitis sufferers have inflammation of *Chlamydia trachomatis* origin, they require immune protection against Chlamydial infection. We are published the biotechnological properties of an immunogen conjugated cultural vaccine used for chronic prostatitis sufferers. The vaccine was prepared from *Chlamydophila psittaci*. The antigenic properties of *Chlamydia trachomatis* and *Chlamydophila psittaci* have similar genus-specific antigenes. Since *Chlamydia trachomatis* has toxicity and is not immunogenic, we used our own isolated strain of *Chlamydophila psittaci*. To eliminate infectivity, we modified the strain using ultraviolet (UV) radiation. To increase immunogenicity, we used a biopeptide, the amino acid methionine. To confirm Chlamidial origin of inflammation, we cultured samples from chronic prostatitis sufferers, including: expressed prostatic secretion (EPS), sperm and urethral swab. Chlamydia was isolated in cell culture: McCoy, NCTC L-929. After confirming the cause of inflammation, it was necessary to test each sufferer's lymphocyte sensitivity to Chlamydial antigene. If lymphocyte inhibition is more than 8%, desensitization of the sufferer's body is necessary first. Antibody levels may be found in chronic prostatitis sufferers with either no history of chlamydial infection or those with past infection. A single specimen endpoint titer of 1:64 to 1:512 should be considered evidence of infection at an undetermined time. Antibodies against Chlamydia can persist for a long time, sometimes month or even years, yet this does not indicate an active infection. Chlamydia antibody detected decreases with time since infection, most notable in the first six months.

The aim. To desensitize chronic prostatitis sufferer's bodies to *Chlamydia trachmatis* and to increase specific *Chlamydial* protective antibody titers in the blood after desensitization.

Material and Methods. To detect Chlamydial cause of inflammation, it was necessary to isolate live *Chlamydia trachomatis* from sufferer's samples. This was accomplished using cell culture: McCoy, NCTC L-929, where they were grown. For research, cell culture was kindly provided by the Cell Bank of Vertebrates from Doctor of Biological Science Galina Poljanskaya at the Research Institute of Cytology.

The *Chlamydia trachomatis* was also cultivated in Flech-385/13 cell culture, kindly provided by the Cell Bank of the Laboratory of Cell Culture from Tatyana Smirnova at the Research Institute of Grippe. Conjugated Chlamydial vaccine was produced using Flech-385/13 cell culture at passage twelve.

Cultural conjugated Chlamydial vaccine produced from *Chlamydophila psittaci* has a titer of infectivity of 1:32 in reaction bind of complement (RBC). For desensitization, 1.66 x 10^6 cells/ml (elementary bodies) were necessary. This is three times diluted from pure vaccine. Before starting desensitization, it was necessary to

obtain blood test results of immune status, where sensitivity of lymphocytes to Chlamydial antigen was tested beforehand.

The research included 25 men of reproductive age (18-45 years old) after obtaining their consent, who had previously been tested positive for Chlamydial infection.

Before to starting desensitization with the Chlamydial conjugate, it was necessary to test basic components of the immune system and sensitivity of lymphocytes to chlamydial antigen. Cellular and humoral components of the immune system were tested in the Laboratory of Immunology at the Research Institute of Eye Disease and Tissue Therapy, Ukraine. Titration of chlamydial antigen showed in reaction bind of complement (RBC) 1:32, for desensitization, it was necessary to dilute conjugated vaccine by three times 1:3.

Results and Discussion: The diluted vaccine must be injected strictly intradermally in doses of 0.1 ml. This dose is allocated in 3-4 places on ther forearm. The body has a memory for Chlamydial antigen. Therefore, every subsequent day, the dose is increased by 0.1 ml intradermally but also allocated intradermally in 4-6 places on the forearm. The course of desensitization strictly requires 8 (eight) intradermal injections in the forearm. It will be possibly to repeat desensitization after 12 months. Chlamydial antigen concentration and dosage are chosen depending on the activity, severity, prevalence, and localization of the inflammatory process.

Table 1

Dilution and injections of Chlamydial vaccine:

№	Titer of Chlamydial antigene by RBC	Concentration of elementary bodies of Chlamydia
1.	1:32 (pure antigen)	3.00 x 10^6 cells/ml
2.	1:64 (diluted twice)	2.50 x 10^6 cells/ml
3.	**1:128** (diluted three time)	1.66 x 10^6 cells/ml
4.	1:256 (diluted four time)	1.25 x 10^6 cells/ml

Dilution of Chlamydial vaccine by three times to a titer of 1:128 contains 1.66 x 10^6 cells/ml elementary bodies of Chlamydia. This is the dose required for desensitization. See table 1.

Table 2

Blood test of prostatitis sufferers M±m, n=25

№	Tested component	Normal range, %	Before desensitization	30 days after desensitiz
1.	Lymphocytes	19-37 %	32.0±1.86	35.0±1.52
2.	Phagocytic activity of neutrophils	40-80 %	68.0±3.64	74.0±2.86
3.	Inhibition of lymphocytes to Chlamydial antigene	up to 8 %	26.0±1,43	20.0±1,14
4.	Titer of antibodies after 30 days of Chlamydia desensitization	5:20	1:20	1:512
5.	Titer of antibodies after 60 days of Chlamydia desensitization	5:20	1:20	1:1024

Before starting desensitization, it is necessary to test basic components of the immune system and sensitivity of lymphocytes to chlamydial antigene.

Chlamydial infection spread through sexual contact, can cause prostatitis.

Before desensitization, inhibition of lymphocytes to Chlamydia antigene was high, three times compared with the normal range, up to 26%. This high sensitivity to Chlamydial antigen indicates sensitization to Chlamydial antigen.

Two months after desensitization, titers of specific antibodies continued to increase. The test of lymphocytes inhibition to Chlamydial antigen started to decrease, taking 2 to 4 months after desensitization. Lymphocyte inhibition to Chlamydial antigen began decreasing, see table 2.

Table 3

Morphological test of EPS of prostatitis sufferers, M±m, n=25

№	Tested component	Normal result	Before desensitization	30 days after desensitization
1.	White blood cells	Up to 10	16±2.36	8±1.64
2.	Red blood cells	0	5±1.73	0
3.	Macrophages	0	7±1.48	2±0.37
4.	Lecithin grains	60-120	46±3.14	94±4.81
5.	Amyloid bodies	4-8	18±2.32	7±1.35
6.	Cylindrical epithelium	2-4	14±2.74	3±0.84

Morphological tests showed, as indicated in table 3, that Chlamydial inflammation increased white blood cells in the expressed prostatic secretion (EPS), red blood cells, macrophages, amyloid cells, and cylindric epithelium count in EPS and decrease count of lecitin grains. After one month of desensitization with Chlamydial conjugate, prostatic condition showed significant improvement. White blood cells decreased to normal amount, red blood cells were not detected in EPS, macrophages were few, lecitin grains significantly increased, cylimdric epithelium decresed, and amyloid bodies decreased to normal range.

Conclusions. For detection and isolation of *Chlamydia trachomatis*, we used cell cultures McCoy and NCTC L-929. To prepare chlamidial conjugated vaccine, we used strain *Chlamydophila psittaci* cultured in Flech-385/13. For desensitization, we used chlamydial conjugated vaccine. Before desensitization, the morphological condition of the prostate was inflamed. Thirty days after desensitization, titers of chlamydial antibodies increased. Inhibition of lymphocytes to chlamydial antigen decresed. Morphological condition of the prostate normalized. In rare cases, Chlamydial bacteria can spread to the prostate gland. *Chlamydia trachomatis* serum antibody investigation in men is not useful in detection chlamydial genital infections. Methods have been recommended to circumvent this dilemma: antibody tests that clearly differentiate between different Chlamydia species. The most effective solution is to culture samples directly from the expressed prostatic secretion (EPS), sperm, urine, or urethral (via uethral swab) of prostatitis suffering patients.

Cell culture sensitive to Chlamydia strains are available for cultivation on McCoy and NCTC L-929 cell lines and show cytopathic effect in cell culture, demonstrating destruction of cells.

Key words: chronic prostatitis, desensitization, chlamydial vaccine, cell culture.