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NEW MONOCLONAL ANTIBODIES TO THE *Chlamydia trachomatis* MAIN OUTER MEMBRANE PROTEIN AND THEIR IMMUNOBIOLOGICAL PROPERTIES

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One of the methods that have been widely used in the diagnosis of urogenital chlamydia is an enzyme-linked immunosorbent assay (ELISA), the use of which allows for differential diagnosis. In order to increase the efficiency of ELISA test kits production, for the kits for the diagnosis of urogenital chlamydia, based on the principle of indirect modification, following synthetic positive controls (PCs) can be used: a conjugate of IgM (IgA) normal immunoglobulins and monoclonal antibodies (McAbs) to C. trachomatis major outer membrane protein (MOMP). The goal of this work was to obtain high active and affinity McAbs to the C. trachomatis MOMP as well as the study of its immunobiological properties which are important for future biochemical approaches. The study was conducted using: polyclonal antibodies (PcAbs) to C. trachomatis; recombinant major outer membrane protein (MOMP) (191-354 a.r.; W4-W5); epitope mapping based on phage display technology. The original set from 16 clones of hybridomas, producers of McAbs to the C. trachomatis MOMP has been obtained. More than half of the tested McAbs (8 out of 14) were characterized by a rather high titer (≥1:800), and three of them had a titer of ≥1:1600. In general, the McAbs titer was correlated with the value of the affinity constant: McAbs with higher titles were characterized by a high value of the affinity constant. For McAbs with a titer of <1:800, the average K_a is $5.2{\times}10^9$ M^{-1}, while for McAbs with a titer ≥1:800 – K_a = $10.7{\times}10^9$ M^{-1}. Antigenic determinants of two McAbs 293F4 and 291F8 that actively competed with PcAbs are represented by two linear sequences of 320-325 a.r. and 326-330 a.r., respectively. The epitope, which interacts with McAb 296G2, is represented by a linear sequence of 347-352 a.r. McAb 296G2 did not show active competition with serum PcAbs. The resulting set of data allows selecting McAbs for use in PCs of the ELISA kit for the detection of IgA or IgM antibodies to C. trachomatis.

K e y w o r d s: monoclonal antibodies, ELISA, Chlamydia trachomatis MOMP, epitope mapping.

Urogenital chlamydia is one of the most common sexually transmitted infections. According to the World Health Organization, *Chlamydia trachomatis* infects about 90 million people each year by sexual transmission. In Ukraine, the rate of chlamydial urogenital infection is 80 cases per 100,000 population. Nearly 16% of pregnant women are infected with *C. trachomatis*. About 50-60% of tubular infertility cases are caused by chlamydial infection. A quarter of all ophthalmic and respiratory diseases in newborn and younger children are associated with chlamydial infection [1, 2]. The major outer membrane protein (MOMP) plays the most important role for a serological diagnostic purpose among all *C. trachomatis* antigens. This pathogen protein is considered the primary site for interaction with T cells, specific depending on the serovar of microorganisms. MOMP is a transmembrane porin, parts of which are localized on the surface of the pathogen cell [1, 2]

Effective diagnosis is one of important components of urogenital chlamydia control. One of the commonly used methods in diagnostics is enzyme-linked immunosorbent assay (ELISA) that allows

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differential diagnosis – determination of disease stage and course, which is especially important in chronic conditions. For that purpose, blood serum (plasma) and human biological fluids are tested for the presence of IgM, IgA and IgG classes of specific antibodies to pathogen's antigens [3, 4].

As usual ELISA kits for the detection of specific IgM and IgA antibodies to *C. trachomatis* in human serum (plasma) based on the principle of indirect modification. Obtaining positive controls (PCs) is a significant problem in the production of diagnostic kits intended for the detection of IgA and IgM specific antibodies against different pathogens. The classic version of PC is the serum (plasma) of human blood containing specific antibodies of a certain class. However, the frequency of detection of IgA- and IgM-positive sera is negligible. Extremely scarce is the relevant biological material as a raw material for PC obtaining. This circumstance can significantly limit the production of diagnostic kits, especially in the context of large-scale production. In order to increase the efficiency of ELISA test kits production, we proposed a methodological approach that addresses the use of synthetic PCs: for the ELISA test kit for the diagnosis of urogenital chlamydia, based on the principle of indirect ELISA, – the use of a conjugate of IgM (IgA) normal immunoglobulins and monoclonal antibodies (McAbs) to *C. trachomatis* major outer membrane protein (MOMP) [4].

The aim of this work was to obtain high active and affinity McAbs to the *C. trachomatis* MOMP as well as the study of its immunobiological properties which are important for future biochemical approaches.

Materials and Methods

Immunization of animals. An immunization schema for Balb/c mice was chosen according to available literature data and own experience [4-9]. The most effective immune response is usually developed following immunization by antigen mixed with Freund's complete adjuvant (FCA) into hind leg pads. The antigen – recombinant protein, a complete homologue of region 191-354 a.r. (W4-W5) of the *C. trachomatis* main outer membrane protein (MOMP) (Virogen, USA) (the protein is synthesized in *E. coli*, at the C-terminus the protein contains the His_6 fragment). Immunization of the Balb/c mice was carried out subcutaneous in the hind leg pads of mice in a total dose of 70-80 μg per animal. The first two injections were made with Freund's complete adjuvant (FCA), and the third one – without adjuvant. The first three injections lasted for a week. Three months after the start of immunization, an intravenous booster dose of antigen (70-80 μg) was injected intravenously, and on the third day, hybridization of the splenocytes from the mouse spleen with the myeloma cells Sp 2/0 was performed. Thus, the total dose of antigen was 140-160 μg per animal.

Obtaining of hybridomas. The fusion was made using polyethylene glycol 3500-3700 (Sigma, USA) according to Kohler's & Milstein's approach [10] modified by Lane & Koprowski [11]. The hybridoma clones obtained were multiplied on peritoneal macrophage feeder cells in a complete growth H-Y medium (Sigma, USA) supplemented with calf embryo serum (Sigma, USA) and HAT medium (Sigma, USA). The cells were cultivated in 96-well plates for tissue cultures (Costar, USA).

The presence of McAbs in hybridoma growth media was controlled by an indirect ELISA approach on the 10th-12th day of cultivation. Following experiment, the optical density values of supernatant fluids of McAbs-positive cultures were by 2-3 times higher compared to the conjugate. Cells from appropriate wells were taken into 24-well plates with peritoneal macrophage feeder cells in the complete growth medium supplemented with the HT medium (Sigma, USA). The cells obtained were frozen in a medium containing newborn calf serum (50%, Sigma, USA), DMEM medium (43%, Sigma, USA), and dimethylsulfoxide (7%, Sigma, USA). The aliquots of culture fluids were used to determine the McAbs specificity, their titers and affinity constants as well as their isotypes. The McAbs specificity was evaluated by an indirect ELISA approach with recombinant antigen. The hybridomas secreting the most active McAbs of the highest affinity having demonstrated positive results were taken for the next work. The chosen hybridoma clones were then thawed and cloned several times to reach stable levels of antibody synthesis. The cloning was carried out using the end-point dilution method; the hybridoma cells were grown on peritoneal macrophage feeder cells in the complete growth medium.

Hybridoma cells with stabile antibody production were multiplied and injected to mice in order to obtain ascite fluids, the mice having been previously primed by pristane (Sigma, USA). The MAb purification from ascite fluids was carried out by a double precipitation protocol using 18% and 16% Na_2SO_4 (w/v) [7]. The McAb preparations obtained in such

way were taken for the synthesis of horse-radish peroxidase (HRP) containing conjugates.

Indirect ELISA. The sorption of MOMP was made overnight in 0.05 M carbonate-bicarbonate buffer (pH 9.6) at 4 °C, their concentrations being 2.5 mg/ml. For plate washing, a phosphate salt buffer supplemented with the Tween 20 (0.05%) (PBS-T, pH 7.2) was used. The plates were incubated with hybridoma culture medium during 1 h (37 °C) and then washed. To detect the bound antibodies, goat HRP-conjugated anti-mouse immunoglobulins were taken. Immunoenzyme conjugate was added to the wells, incubated during 1 h at the ambient temperature and washed away (three times by the PBS-T and once by water). In all experiments, 0.003 % hydrogen peroxide in 0.15 M citrate buffer (pH 5.0) was used as a substrate, and 3,3′,5,5′-tetramethylbenzidine was a chromogen. The reaction was stopped by 2 M sulphuric acid. The optical density values were read at wavelengths 450/620 nm by a spectrophotometer.

Evaluation of McAbs affinity. The McAb affinity constants were evaluated according to the inhibition method proposed by B. Friguet [12], modified by B. Kim [13]. Affinity constants were calculated by S. Bobrovnik recommendation [14]. Immunoglobulin solutions with different MOMP concentrations (10-9-10-6 mole per litter) were mixed with McAbs-containing culture fluid samples. Following incubation (1 hour at 37 °C) the mixtures were put into 96-well plate wells sensitized previously by MOMP preparation and the ELISA performance was carried out. Control plate wells contained culture fluid samples non-incubated with the MOMP preparation.

Determination of McAbs isotype. The isotype determination of the McAbs obtained was made using a standard kit for this procedure – ISO-2 (Sigma, USA). The isotyping was carried out by antigen-mediated ELISA. Hybridoma culture fluids were put into the plate wells sensitized by MOMP, each sample was put into six wells. The McAbs isotype was determined by a monospecific goat serum. The typing antibodies were detected by an anti-goat HRP-containing conjugate (Sigma, USA). The results were read according to the manufacturer's recommendations.

Epitope mapping. The procedure was based on the algorithm described by Rechkina et al. [15]. The following modification of the ELISA method was used to evaluate the specificity of binding of phages with McAbs to MOMP. McAbs were sorted by 96-well plates at a concentration of 2 μg/ml for 12 h at 4 °C (carbonate buffer solution, pH 8.6, was used for immobilization). After incubation with the blocking buffer to reduce the possible nonspecific binding and 6-fold washings, PBS-T (pH 7.2), was applied to the wells by phage clones at a concentration of 10^{10} plaque-forming unit (PFU) per ml, incubated for 1 h at room temperature. After washing in a hole, conjugated horseradish peroxidase was introduced to polyclonal antibodies against bacteriophage M13 pIII. The next procedure was carried out similarly to the indirect ELISA protocol.

Bioethical norms. The research was conducted in accordance with international and national bioethical recommendations.

Results and Discussion

As previously noted immunization of the Balb/c mice was carried out with recombinant protein – a complete homologue of the 191-354 a.r. (W4-W5) of the *C. trachomatis* main outer membrane protein. As a result of hybridization of mouse splenocytes about 400 clones were obtained. In the initial testing after hybridization, the specific antibodies to the MOMP were detected in all wells of seven tablets. Cultural fluids during the screening were diluted several times, which allowed to significantly reducing the background signal. Thus, 18 clones that had the highest signals according to the results of indirect ELISA were selected. All selected hybridomas were cryopreserved, and culture fluids were left for further study. After repeated testing in ELISA, high activity of the McAbs was confirmed in 16 clones (Table).

At the next stage of the work, in-depth study of the properties of the McAbs was carried out on the following characteristics: the titer of antibodies in the culture liquid, affinity and their isotype. Characteristics of the McAbs for the first two criteria (titer and affinity) would enable to assess the prospects of the use of such McAbs in the immunoassay. The isotype of antibodies, on the one hand, affects the feasibility of using McAbs in various bioanalytical methods, and, on the other hand, is an important prerequisite for choosing of McAbs isolation and purification methods.

The results of determination of the isotype of 16 obtained McAbs are presented in Table. The titer in the culture fluid, as well as the affinity constant, were determined for all McAbs, with the exception of those having IgM isotype (294G2, 296H9), due to adverse conditions of purification and possible

Characteristics of the McAb panel to the C. trachomatis MOMP

McAbs	Optical density[1)] in ELISA	Isotype	Titer in cultural fluids[1)]	Affinity constant[1)], 10^9 M-1	Competition with PcAbs	Antigenic determinant
291B2	2.588	IgG_{2a}	1:800	16.0	–[2)]	n/d[3)]
291C10	2.440	IgG_{2b}	1:400	4.0	n/d	n/d
291F8	2.805	IgG_{2a}	1:1600	16.0	+[2)]	(326)VLDVT(330)
293C8	2.496	IgG_1	1:800	8.0	–	n/d
293D2	2.402	IgG_1	1:800	8.0	–	n/d
293F4	2.789	IgG_{2a}	1:1600	16.0	++	(320)TTLNPT(325)
294F5	2.602	IgG_{2b}	1:800	8.0	+	n/d
294F7	2.214	IgG_1	1:400	4.0	n/d	n/d
294G2	2.066	IgM	n/d	n/d	n/d	n/d
295A5	2.188	IgG_{2a}	1:200	2.0	n/d	n/d
295D2	2.661	IgG_1	1:400	8.0	n/d	n/d
296A10	2.177	IgG_{2a}	1:400	8,0	n/d	n/d
296B11	2.407	IgG_{2a}	1:800	8.0	+	n/d
296G2	2.890	IgG_{2a}	1:1600	16.0	–	(347)IVSLQL(352)
296H8	2.224	IgG_{2a}	1:800	16.0	+	n/d
296H9	2.18	IgM	n/d	n/d	n/d	n/d

Notes. [1)] The mean values of the values based on the results of the study of supernatants with a hybrid in 4 replicates are given ($P < 0.05$). [2)] "+" McAbs compete with PcAbs; "–" McAbs' competition with PcAbs was not found. [3)] Parameter not defined.

cross-activity. More than half of the tested McAbs (8 out of 14) were characterized by a rather high titer (≥1:800), and three of them (291F8, 293F4, 296G2) had a titer of ≥1:1600. It should be noted that in general, the McAbs titer was correlated with the value of the affinity constant: McAbs with higher titles were characterized by a high value of the affinity constant. For McAbs with a titer of <1:800, the average K_a is 5.2×10^9 M^{-1}, while for McAbs with a titer ≥1:800 – $K_a = 10.7\times10^9$ M^{-1}.

Altogether of obtained data (ELISA activity, isotype, titer, and McAbs' constant affinity) was used to select the clones with the hybrid for their further defrosting, cloning, and accumulation of antibodies. Preference was given to clones with high titers (≥1:800) and affinity constant ($\geq 8.0\times10^9$ M^{-1}), as well as an intense signal in indirect ELISA. The following McAbs corresponded to selected criteria: 291B2, 291F8, 293C8, 293D2, 293F4, 294F5, 296B11, 296G2, 296H8. Cloning of hybridomas was performed by limiting dilutions technique. All clones were cloned 1 to 3 times – up to almost complete stability at the level of synthesis of specific McAbs. Isolated positive clones from a 96-well plate were transplanted into 24-well, hybridomas were grown, frozen and administered to the mice intraperitoneally. At 7-10 days the animals accumulated ascites, which were taken. One mouse gave up to 10 ml of ascitic fluid on average. After isolation from ascitic fluid, McAbs was used for the synthesis of peroxidase conjugates that were needed to establish their epitope specificity.

In order to establish the epitope specificity of McAbs, we can use different methodological approaches. One of them is a comparative epitope specificity technique. We have used such approach many times [4, 5, 16] when different bioanalytical methods were developed (ELISA for determination of human IgE, IgM, and IgA). In these cases, absolute epitopic specificity is not important; for

the development of the analytical methods, it was important to understand the ratio of the specificity of various antibodies. Another principle is addressed to determine an antibody specific binding place (amino acid sequence) on an antigen surface (epitope) [14, 16]. When we are talking about anti-MOMP antibodies, it is necessary to understand its future application. Obtained McAbs are intended for the synthesis of synthetic (hybrid) positive controls of ELISA kits for diagnosing urogenital chlamydia. In this case, anti-MOMP monoclonal antibodies (in a conjugate with normal human immunoglobulins) should effectively interact with an antigen sorbed on a solid phase (immunosorbent). At the same time, during ELISA staging immobilized antigen interacts with specific anti-*C. trachomati*s polyclonal antibodies (PcAbs) of human blood serum. Due to this circumstance, it will be expedient to analyze the molecular-cellular localization of the MOMP in the outer membrane of the pathogen of urogenital chlamydia and the potential orientation of the anti-MOMP immune response of the macroorganism. Based on the schematic representation of the MOMP polypeptide chain of *C. trachomatis* serovar F (Fig. 1) [17], it is understandable that only certain part of the polypeptide chain (the part that is located above the membrane) is available for protective polyclonal antibodies present in blood serum of infected individuals.

However, one cannot reject the probability of presence in human blood serum of the PcAbs, which recognizes other ("internal") antigenic determinants of the MOMP molecule. Such antibodies probably do not have to be protective, highly affiliated and the main content of the whole repertoire of PcAbs. Thus, in this case, the localization of the antigenic determinant to which the monoclonal antibody is directed (the outer part of the MOMP polypeptide chain, a part in the transmembrane space or in the periplasm) may be important.

To evaluate the spatial localization of antigenic determinants to which the McAbs were directed, we have used competitive ELISA with PcAbs that are specific to the *C. trachomatis* MOMP. To implement this approach, it was necessary previously to select specific anti-MOMP polyclonal antibodies from blood serum from *C. trachomati*s infected individuals. For the solution of this problem at the preliminary stage of work a pool of specific PcAbs was obtained, which was used to formulate a competi-

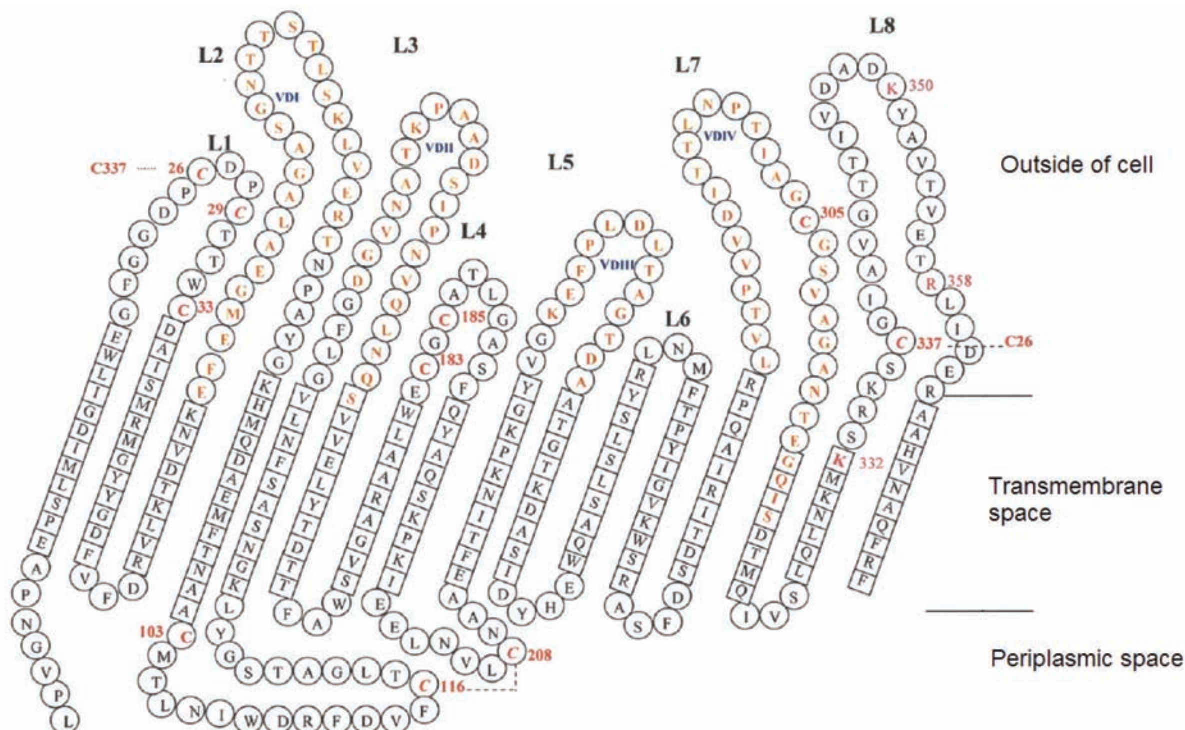

Fig. 1. Schematic representation of the placement of the C. trachomatis MOMP polypeptide chain serovar F in the outer membrane (the letters indicate the amino acid residues; the hinges are indicated (L); the place of formation of disulfide bonds is indicated by dotted lines) [17]

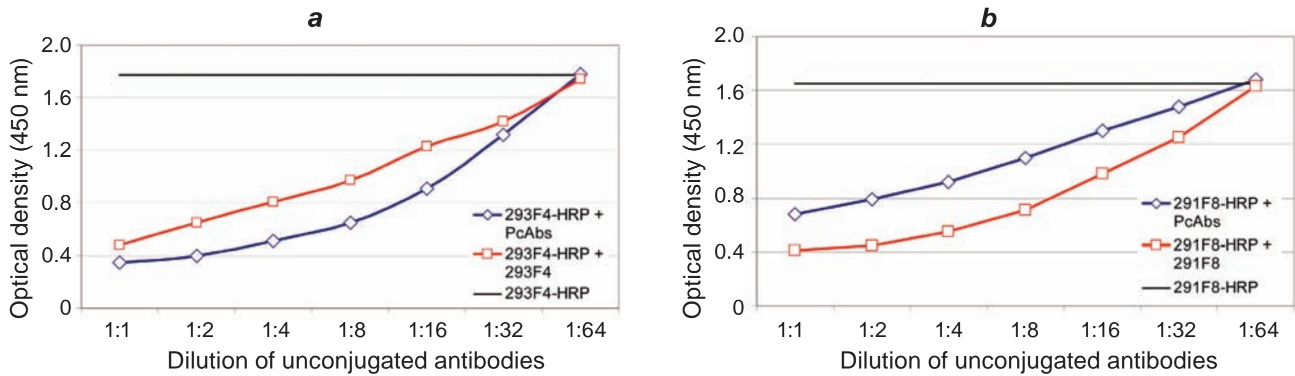

*Fig. 2. The competition profile of McAbs 291F8 (**a**) and 293F4 (**b**) with PcAbs from blood serum of C. trachomatis infected persons (calculation of the mean value of optical density for each dilution was performed by the results of ELISA in 4 replies, P < 1%)*

tive ELISA (studying the PcAbs ability to compete with peroxidase conjugates of different McAbs for binding of antigen to the solid phase). The results of the corresponding experiments for some McAbs are presented in Fig. 2-3.

Among the 9 tested McAbs 4 antibodies did not compete with serum polyclonal antibodies (291B2, 293C8, 293D2, 296G2), and the remaining 5 clones, on the contrary, competed for the site of binding to the antigen with PcAbs (291F8, 293F4, 294F5, 296B11, 296H8) (Table). Thus, among the received McAbs panel it was presented antibodies of both possible groups of specificity: directed toward the external epitopes of the *C. trachomatis* MOMP and formed by amino acid residues (a.r.) localized in the periplasmic and/or transmembrane space. Among the most active and highly affiliated McAbs were clones of both variants of epitope specificity. For a more in-depth characterization of those McAbs (291F8, 293F4, 296G2), an additional series of work was carried out to determine their absolute epitope specificity with the use of phage display technology.

As already have noted, the technology of the phage display implies the use of filamentous phages, on the surface of which whimsical proteins containing amino acid sequences as potential antigenic determinants are expressed [16]. Within the framework of this block of research, by the results of DNA sequencing we have determined the amino acid sequence of the peptide phage insert, denoting a.r. by means one-letter code.

To find the motifs, which are homological to phage peptide, a comparative analysis of the amino acid sequences of the peptide inserts and the *C. trachomatis* MOMP (Protein Database, NCBI) has been done. It should be noted that in the analysis, analogous as well as homologous a.r. were considered (they were grouped according to their physical and chemical properties [15, 18-20]), namely:

- polar charged positively: R (Arg) = K (Lys) = H (His);
- polar charged negatively E (Glu) = D (Asp);
- polar uncharged with compensated charges: S (Ser) = T (Thr) and Q (Gln) = N (Asn);
- hydrophobic: L (Leu) = V (Val) = I (Ile) = M (Met);
- nonpolar with small radicals: A (Ala) = G (Gly);
- nonpolar with large radicals containing the aromatic ring: W (Trp) = F (Phe) = Y (Tyr).

Association of a.r. to such groups is also important in terms of their potential role in the formation of antigenic determinants. The works of various authors point to the influence of different groups of a.r. on the binding of antigenic determinants. For exam-

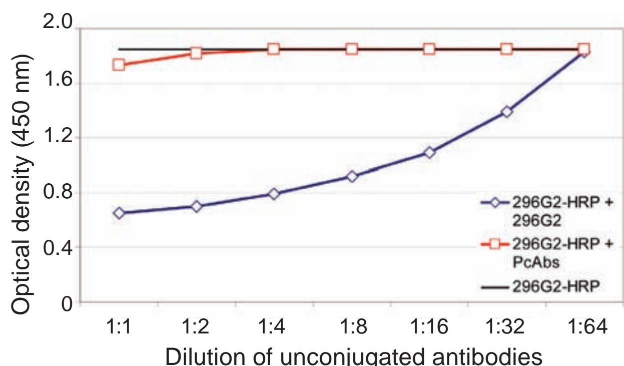

Fig. 3. The competition profile of McAbs 296G2 with PcAbs from blood serum of C. trachomatis infected persons (calculation of the mean value of optical density for each dilution was performed by the results of ELISA in 4 replies, P < 1%)

ple, the role of charged a.r. in the formation of the epitopes of anti-HIV proteins and tyrosine kinase receptor of the epidermal growth factor [18, 19], the participation of hydrophobic nonpolar a.r. in the formation of antigenic determinants of the core protein of the hepatitis C virus [20].

It should be noted that the immunogen used in our work to produce anti-MOMP McAbs was a recombinant protein – a complete homologue of 191-354 a.r. (W4-W5) of native protein. The choice of this recombinant protein for immunization of animals was due to the results of the analysis of literature data on the study of the humoral immune response against *C. trachomatis* MOMP: various authors report that the site 191-354 a.r. is the most immunogenic and provides the strongest immune response to the causative agent of urogenital chlamydia, in particular a.r. of this site forms the species-specific and serovar-specific epitopes [21-25].

After three rounds of affinity selection from the non-amplified elutes of the last round 11 phage clones (McAb 293F4), 10 phage clones (McAb 291F8) and 12 phage clones (McAb 296G2) were identified (and the sequence of insertions was determined) (Fig. 2). Alignment of the obtained peptides from MOMP *C. trachomatis* allowed locating the specified a.r.

Consequently, the epitope that interacts with McAb 293F4 is localized on a site 320-325 a.r. in the form of "TTLNPT" sequence. It is also worth noting the significance of a.r. at 323rd place: in almost all clones there is an amino acid residue of asparagine N (Asn) at this site, but in several clones in this position there is glutamine D (Asp). Asparagine and aspartic acid have approximately the same size of the side radical, although somewhat differ in charge of the radical. Consequently, this position is also important for binding of the antibody. Thus, this antigenic determinant forms by 4 polar a.r. (T, N) and 2 nonpolar a.r. (P, L). The sequence (320)TTLNPT(325) is part of L7 loop (in native form it is located above the transmembrane space of the microorganism).

The antigenic determinant that binds to McAb 291F8 is localized at the site 326-330 a.r. in the form of "VLDVT" sequence. Amino acid residues that form this epitope, also refer to both polar (D, T) and nonpolar (V, L). This sequence is also part of L7 loop.

The epitope that interacts with the paratope of McAb 296G2 is localized on the site 347-352 a.r. in the form of "IVSLQL" sequence. Note that a.r. at 348th position has a certain feature: in almost all clones there is an amino acid residue of serine S (Ser) on this site, but in the 2 clones in this position there is cysteine C (Cys). Serine and cysteine have approximately the same size and charge of the side radical. Such data indicate the importance of this a.r. for the formation of antigenic determinants and binding of a paratope. The hexapeptide (347) IVSLQL(352) containing 4 nonpolar (I, V, L) and 2 polar (S, Q) a.r. in the native molecule of MOMP is located both in the periplasmic (IVS) and in the transmembrane (LQL) spaces. It is evident that the formation of such antigenic determinants is possible through appropriate antigen-presenting cell antigen processing. It should be noted that the results of the establishment of the absolute epitope specificity of McAbs 293F4, 291F8, and 296G2 completely coincide with the results of the competition of these McAbs in the ELISA with the polyclonal antibodies.

Thus, an original panel of 9 high-level and affinity monoclonal antibodies to the *C. trachomatis* MOMP was obtained. The study of their biological properties (constant affinity, titer in the culture liquid, isotype) was carried out. The ability of 5 McAbs of the received panel to compete with polyclonal antibodies of serum of *C. trachomatis* infected persons was determined. It may specify the potential protective activity of these antibodies. However, 2 McAbs did not exhibit significant competition with polyclonal antibodies. For the three most affinity monoclonal antibodies, an absolute epitope specificity was determined using phage display technology.

Antigenic determinants of two McAbs 293F4 and 291F8 that actively competed with PcAbs are represented by two linear sequences of 320-325 a.r. and 326-330 a.r., respectively. This portion of the polypeptide chain in the native MOMP molecule is located above the plasma membrane and is involved in the formation of L7 loop. The epitope, which interacts with McAb 296G2, is represented by a linear sequence of 347-352 a.r. (in the native MOMP molecule it is located both in the periplasmic and in the transmembrane space). McAb 296G2 did not show active competition with serum PcAbs. The resulting set of data allows selecting McAbs for use in hybrid (synthetic) positive control of the ELISA kit for the detection of IgA or IgM antibodies to the urogenital chlamydial pathogen.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

НОВІ МОНОКЛОНАЛЬНІ АНТИТІЛА ДО ОСНОВНОГО ПРОТЕЇНУ ЗОВНІШНЬОЇ МЕМБРАНИ *Chlamydia trachomatis* ТА ЇХНІ ІМУНОБІОЛОГІЧНІ ВЛАСТИВОСТІ

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Одним із методів, який знайшов широке застосування в діагностиці урогенітального хламідіозу, є імуноензимний аналіз (ІЕА), застосування якого дозволяє проводити диференціальну діагностику. Для підвищення ефективності виробництва ІЕА наборів, тих, що призначені для діагностики урогенітального хламідіозу та побудовані за принципом непрямого аналізу, можна застосовувати такі синтетичні позитивні контролі (ПК): кон'югати нормальних імуноглобулінів IgM (IgA) з моноклональними антитілами (McAbs) до основного протеїну зовнішньої мембрани *Chlamydia trachomatis* (MOMP).

Метою цієї роботи було одержання високоактивних та специфічних McAbs до *C. trachomatis* MOMP, а також вивчення їхніх імунобіологічних властивостей, важливих для майбутнього біохімічного застосування.

Дослідження проводили з використанням: поліклональних антитіл (PcAb) до *C. trachomatis*; рекомбінантного основного зовнішнього мембранного протеїну (MOMP) (191-354 а.з.; W4-W5); епітопного картування на основі технології фагового дисплея.

Було одержано оригінальний набір із 16 клонів гібридоми, продуцентів McAb до *C. trachomatis* MOMP. Більше половини досліджених McAb (8 із 14) характеризувалися доволі високим титром (≥ 1:800), а три з них мали титр ≥ 1:1600. Загалом титр McAb корелював зі значенням константи афінності: McAb із вищим титром характеризувалися високим значенням константи афінності. Для McAb із титром < 1:800 середнє значення K_a становило $5{,}2\times10^9$ M^{-1}, у той час як для McAb із титром ≥ 1:800 – K_a дорівнювало $10{,}7\times10^9$ M^{-1}. Встановлено здатність п'яти McAb одержаної панелі конкурувати з поліклональними антитілами сироватки осіб, інфікованих *C. trachomatis*, що може обумовлювати потенційну протективну активність цих антитіл. Разом із тим, два McAb не виявляли вираженої конкуренції з поліклональними антитілами. Для трьох найбільш афінних моноклональних антитіл проведено визначення абсолютної епітопної специфічності з використанням технології фагового дисплея. Антигенні детермінанти двох McAb 293F4 та 291F8, що активно конкурували із PcAb, представлено двома лінійними послідовностями 320-325 а.з. та 326-330 а.з. відповідно. Епітоп, з яким взаємодіє McAb 296G2, представлений лінійною послідовністю 347-352 а.з. McAb 296G2 не виявляло активної конкуренції щодо PcAb сироватки. Одержана сукупність даних дає змогу обрати McAb для використання у ПК ІЕА-наборів для виявлення IgA/IgM-антитіл до *C. trachomatis*.

К л ю ч о в і с л о в а: моноклональні антитіла, імуноензимний аналіз, *Chlamydia trachomatis* MOMP, епітопне картування.

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