39. Mueller S. Poliovirus and poliomyelitis: A tale of guts, brains, and an accidental event / S. Mueller, E. Wimmer, J. Cello // Virus Research. – 2005. – Vol. 111, № 2. – P. 175-193.
40. Centers for Disease Control and Prevention. Poliomyelitis prevention in the United States: introduction of a sequential vaccination schedule of inactivated poliovirus vaccine followed by oral poliovirus vaccine; recommendations of the advisory committee on immunization practices (ACIP) // Morbid. Mortal. Weekly Rep. – 1997. – Vol. 46. – P. 1-25.
41. Antibody response and viral excretion after live polio vaccine or a combined schedule of live and inactivated polio vaccines / [Ramsay M.E., Begg N.T., Gandhi J. et al.] // Pediatr. Infect. Dis. J. – 1994. – Vol. 13, № 12. – P. 1117-1121.
42. Caldwell M. The Dream Vaccine / M. Caldwell // Discover. – 1997. – Vol. 9. – P. 3-5.

UDC 577.27:57.083.33

OBTAINING OF MONOCLONAL ANTIBODIES TO HUMAN IgG SUITABLE FOR USAGE IN HIGHLY SENSITIVE AND SPECIFIC IMMUNOASSAYS

[1,2]Galkin O.Yu., [3]Solovjova V.F., [1]Metalnikova N.P., [1]Dugan O.M., [1]Gorgo Yu.P.

[1]National Technical University of Ukraine "Kyiv Polytechnic Institute"
03056, Ukraine, Kyiv, Peremogy av., 37, [2]Hema Ltd.
03022, Ukraine, Kyiv, Vasilkovskaya str. 98
[3]Ukrainian Scientific & Research Institute of Nutrition, Biotechnology and Pharmacy
01042, Ukraine, Kyiv, Chygorina str., 18

alexfbt@mail.ua

The original set from 12 clones of hybridomas, producers of monoclonal antibodies (McAbs) against human IgG has been obtained. Criteria for selecting McAbs with satisfactory properties has been justified. Monoclonal antibodies with such properties have to provide high informativeness indexes (sensitivity and specificity) of immunoassay methods based on obtained McAbs. The study of following biological properties of McAbs has been conducted: specificity, constant of affinity and titer in a cultural medium. Obtained McAbs are directed to the two epitop regions on IgG molecule. The first group of McAbs relates to epitop region, represented by two epitopes; the second epitop region is represented by only one epitop.

Keywords: monoclonal antibodies, hybridomas, human IgG, affinity, epitop mapping.

ОТРИМАННЯ МОНОКЛОНАЛЬНИХ АНТИТІЛ ДО IgG ЛЮДИНИ, ПРИДАТНИХ ДЛЯ ВИКОРИСТАННЯ У ВИСОКОЧУТЛИВИХ І СПЕЦИФІЧНИХ МЕТОДАХ ІМУНОАНАЛІЗУ

[1,2]Галкін О.Ю., [3]Соловйова В.Ф., [1]Метальнікова Н.П., [1]Дуган О.М., [1]Горго Ю.П.

[1]Національний технічний університет України «Київський політехнічний інститут»
03056, Україна,Київ, пр. Перемоги, 37

[2]ТОВ «Хема»,
03022, Україна, Київ, вул. Васильківська, 98

[3]Український науково-дослідний інститут харчування, біотехнології та фармації
01042, Україна,Київ, вул. Чигоріна, 18

alexfbt@mail.ua

Одержано оригінальний набір з 12 клонів гібридом, продуцентів моноклональних антитіл (МкАт) до IgG людини. Проведено обґрунтування критеріїв відбору МкАт із властивостями, що забезпечуватимуть високі показники інформативності (чутливості та специфічності) розроблюваних на їх основі методів імуноаналізу. Проведено поглиблене вивчення таких біологічних властивостей антитіл: встановлено їхню специфічність, константу афінності та титр у культуральній рідині. Отримані антитіла спрямовані до двох епітопних регіонів на молекулі IgG.

Перша група антитіл відноситься до епітопного регіону, що представлений двома епітопами; другий епітопний регіон представлений лише одним епітопом.

Ключові слова: моноклональні антитіла, гібридоми, IgG людини, афінність, епітопне картування.

ПОЛУЧЕНИЕ МОНОКЛОНАЛЬНЫХ АНТИТЕЛ К IgG ЧЕЛОВЕКА, ПРИГОДНЫХ ДЛЯ ИСПОЛЬЗОВАНИЯ В ВЫСОКОЧУВСТВИТЕЛЬНЫХ И СПЕЦИФИЧНЫХ МЕТОДАХ ИММУНОАНАЛИЗА

[1,2]Галкин А.Ю., [3]Соловьева В.Ф., [1]Метальникова Н.П., [1]Дуган А.М., [1]Горго Ю.П.

[1]Национальный технический университет Украины «Киевский политехнический институт»
03056, Украина,Киев, пр. Победы, 37

[2]ООО «Хема»
03022, Украина, Киев, ул. Васильковская, 98

[3]Украинский научно-исследовательский институт питания, биотехнологии и фармации
01042, Украина, Киев, ул. Чигорина, 18

alexfbt@mail.ua

Получен оригинальный набор из 12 клонов гибридом, продуцентов моноклональных антител (МкАт) к IgG человека. Проведено обоснование критериев отбора МкАт со свойствами, которые будут обеспечивать высокие показатели информативности (чувствительности и специфичности) разрабатываемых на их основе методов иммуноанализа. Проведено углубленное изучение таких биологических свойств антител: установлена их специфичность, константа аффинности и титр в культуральной жидкости. Полученные антитела направлены к двум эпитопным регионам на молекуле IgG. Первая группа антител относится к эпитопному региону, представленному двумя эпитопами; второй эпитопный регион представлен только одним эпитопом.

Ключевые слова: моноклональные антитела, гибридомы, IgE человека, аффинность, эпитопное картирование.

INTRODUCTION

As known immunoglobulins (Igs) are a group of glycoproteins which are contained in the blood plasma (antibodies) and on the surface of certain cells of immune system (immunoglobulin receptors). There are five classes of human immunoglobulins: IgG, IgA, IgM, IgE, and IgD, which are different in their structure, properties and functions. IgG is the major class of antibody in humans, it constitutes 70-75 % of all immunoglobulins. IgG always play an important role both at the primary and the secondary immune response [1]. Immunoglobulin G is a bifunctional molecule, which is responsible for binding to the antigen and provides some more effector functions (e.g., to activate complement, bind to cells and others). Structural regions of the IgG molecules, which are responsible for effector activity, outlying from the antigen binding sites and are located in the Fc-region. The isotype of Igs are also due to antigenic determinants of Fc-fragment [2].

By the presence of specific IgG in human plasma it's possible to conclude about the presence of the pathogen in the body or about infection in anamnesis. Therefore, diagnosis of many infections are based on the detection of specific IgG in human serum.

Enzyme-linked immunosorbent assay (ELISA) is one of the methods which is widely used in the diagnosis of infectious diseases. This method can be applied to determine the pathogen antigens and detection of antibodies against it. Development and production of ELISA test kits for the diagnosis of these infections are not possible without anti-species antibodies (e.g., mouse Abs against human Igs) specific to a particular class of immunoglobulins [3]. Polyclonal Abs to human Igs have a number of deficiencies (non-specific binding, cross-reactivity, low level of studies results reproducibility, etc.), which significantly limits their usage [4]. More successful reagents in this case are anti-Igs monoclonal antibodies (McAbs). The advantages of McAbs are the exclusive specificity, homogeneity and the possibility of obtaining virtually unlimited quantities. Having armed by panel of McAbs to human immunoglobulines it is possible to select high affinity and specific clones, which will be suitable for usage in highly sensitive and specific immunoassays. It is known that the development of any ELISA test kit needs a wide range (set) of relevant McAbs, because usually only one or several clones from whole McAbs panel provide satisfactory results [3-4].

The aim of our study was: obtaining of monoclonal antibodies to human IgG, study their properties and selection of McAbs which are most promising for use in highly sensitive and specific immunoassay methods.

MATERIALS AND METHODS OF THE STUDY

Immunization of animas. An immunization schema for Balb/c mice was chosen according to available literature data and own experience [5-11]. The most effective immune response is usually developed following immunization by antigen mixed with Freund's complete antigen (FCA) into hind leg pads [12]. The antigen – human IgG used for immunization – was purified in our laboratory. In the course of immunization (during 7-8 days) each animal recieved 50-60 мg of the human IgG preparation. Two first injection were carried out using the FCA, and the last one – without any adjuvant. On the third day following the last antigen injection, lymphocytes were taken from regional lymphatic nodes and fused with myeloma Sp 2/0 cultured cells.

Obtaining of hybridomas. The fusion was made using polyethylene glycol 3500-3700 (Sigma, USA) according to Kohler's & Milstein's approach [13] modified by Lane & Koprowski [14]. The hybridomal clones obtained were multiplied on peritoneal macrophage feeder cells in a complete growth medium H-Y (Sigma, USA) supplemented with calf embryo serum (Sigma, USA) and HAT medium (Sigma, USA). The cells were cultivated in 96-welled plates for tissue cultures (Costar, USA).

The presence of anti-human MAbs in hybridomal 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 comparing to the conjugate. Cells from appropriate wells were taken into 24-welled 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 human different class antibodies – IgA, IgM, and IgG as well as with IgG Fc-fragments. The hybridomas secreting the most active McAbs of the highest affinity having demonstrated positive results with human IgG and their Fc-fragments and showing no cross reactions with any Igs classes were taken for the next work. The chosen hybridomal clones were then thawed and cloned several times to reach stabile levels of antibody synthesis. The cloning was carried out using the end-point dilution method; the hybridomal cells were grown on peritoneal macrophage feeder cells in the complete growth medium. The screening of hybridomal cells was realized by the indirect ELISA approach evaluating the interactions of McAbs secreted by these cells with a human IgG preparation.

Hybridomal 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) [10]. The McAb preparations obtained in such way were taken for synthesis of horse-radish peroxidase (HRP) containing conjugates.

Indirect ELISA. The sorption of human IgG and their Fc-fragments was made overnight in 0,05 M carbonate-bicarbonate buffer (pH 9,6) at 4 °C, their concentrations being 5 and 2,5 мg/ml, respectively. For plate washing, a phosphate salt buffer supplemented with the Tween 20 (0,05 %) (PBS-T, pH 7,2-7,4) was used. The plates were incubated during 1 h (37 °C) and then washed. To detect the bound antibodies, goat HRP-conjugated anti-mouse immunoglobulins were taken. They were 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 substrate, and 3,3',5,5'-tetramethylbenzidine was a chromogen.

The reaction was stopped by 2 M sulfuric acid. The optical density values were read at wave lengths 450/620 nm by spectrophotometer.

Evaluation of McAbs affinity. The McAb affinity constants were evaluated according to the inhibition method proposed by B. Friguet [15] and modified by B. Kim [16]. Affinity constants were calculated by S. Bobrovnik recommendation [17]. Immunoglobulin solutions with different IgG concentrations (10^{-9}-10^{-6} mole per liter) were mixed with McAbs-containing culture fluid samples. Following incubation (1 h at 37 °C) the mixtures were put into 96-welled plate wells sensitized previously by a human IgG preparation and the ELISA performance was carried out. Control plate wells contained culture fluid samples non-incubated with the IgG preparation.

Determination of the 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. Hybridomal culture fluids were put into the plate wells sensitized by human IgG, each sample was put into six wells. The McAbs isotype was determined by a goat monospecific 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.

Synthesis of HRP-containing conjugates. The McAb conjugation with the horse-radish peroxidase was carried out according to periodate oxidation approach [18] with some modifications, the IgG/enzyme mass ratio being 2:1. The HRP (15 mg/ml, Sigma, USA) was diluted in the 0.1 M bicarbonate buffer (pH 8.3) and mixed with an equal volume of the sodium periodate solution (14 мM). For the HPR oxidation, the mixture was incubated during 2 h at the ambient temperature. The solution of the oxidized HRP was mixed with an antibody solution which had been previously dialyzed against the 0.1 bicarbonate buffer (pH 9.2). This mixture was then put into a chromatographic column; dry Sephadex G-25 (Fluka, Switzerland) was added (1:3, v/v), and the mixture was incubated 3 h at the room temperature. The conjugate solution was eluted from the column; 1/20 volume of the aqueous $NaBH_4$ solution (5 mg/ml) was then added. To stop the reaction, the mixture was kept 30 min at room temperature; then additional portion of the $NaBH_4$ solution (3:20, v/v) was added, and the mixture was incubated 60 min. The obtained solution of a McAbs-HPR conjugate was dialyzed against the 0.02 M phosphate buffer containing 0.15 M NaCl.

Studies on animals were carried out in compliance with bioethical norms [19].

RESULTS AND ITS DISCUSSION

More than 900 different hybridomal clones were obtained following hybridization. To choice primary effective clones, culture fluids of hybridomas were tested to determine their interaction with human IgG; the McAbs specificity was assayed with IgG Fc-fragments (Fγc); the McAbs cross-reactivity was assessed testing their interactions with human IgA and IgM to reject cross-reacting hybridomal clones. During the first testing following hybridization, specific antibodies against human IgG were detected in all wells of seven plates. To find the most active hybridomas, culture fluids were diluted for the next screening, the background levels having become significantly lower. In such a way 37 clones were chosen with the highest signals according to the ELISA results. All hybridomes chosen were cryoconserved, their culture fluids having been taken for further investigations. As a result of a repeated testing, high MAbs activities were confirmed for 21 hybridomal clones (table 1).

The next analysis step was to check hybridomal culture fluids and to determine if they possessed the cross-reactivity with human IgM and IgA; we had also to determine our McAbs specificity to human IgG Fc- or Fab-fragment. For such purpose, the titers of all these hybridomal culture fluids were evaluated using human IgG and its Fc-fragments as antigens; results obtained were compared.

The analysis of our results demonstrated the following:

9 clones gave high signals (signal in ELISA ≥ 2.8) while their testing with human IgG and human IgG Fc-fragments showing no cross-reactivity with IgM and IgA molecules (clones 201C8, 202B7, 202D10, 204H7, 206B2, 206C8, 206E3, 207F3, 207G8);

9 clones gave somewhat lower signals (signal in ELISA < 2.8) both with human IgG and its Fc-fragments showing no cross-reactivity with IgM and IgA (clones 201C9, 201F10, 202B11, 203D10, 203G4, 206C12, 206H11, 207B10, 207G4);

3 clones showed higher signals with human IgG comparing to their Fc-fragments or demonstrated their cross-reactivity with human IgM and IgA (clones 201A2, 206H10, 208D5).

Table 1 – Characterization of monoclonal antibodies to human IgG

No	Hybridoma /McAbs	Optical density in ELISA [1)]				Isotype	Titer in cultural liquid [1)]	Affinity constant [1)], 10^9 M^{-1}	Epitope specificity
		IgG	Fγc	IgA	IgM				
1	201A2	2,742	2,777	1,035	0,902	– [2)]	–	–	–
2	***201C8***	3,001	2,985	0,039	0,019	IgG_1	1:800	20.0	A2
3	201C9	2,077	2,139	0,066	0,052	IgG_1	1:500	4.0	–
4	***201F10***	2,889	2,661	0,072	0,070	IgG_1	1:800	10.0	B
5	***202B7***	3,094	3,002	0,048	0,101	IgG_{2a}	1:1000	20.0	A1
6	202B11	2,402	2,201	0,025	0,020	IgG_1	1:400	4.0	–
7	***202D10***	3,112	3,066	0,076	0,010	IgG_{2a}	1:1000	28.0	A1
8	***203D10***	2,145	2,321	0,025	0,066	IgG_1	1:800	20.0	A2
9	***203G4***	2,333	2,451	0,045	0,081	IgG_{2b}	1:800	16.0	B
10	204H7	2,988	2,904	0,081	0,090	IgM	–	–	–
11	***206B2***	3,008	2,988	0,031	0,035	IgG_1	1:800	20.0	A1
12	***206C8***	3,015	3,025	0,029	0,019	IgG_{2b}	1:1000	28.0	A2
13	***206C12***	2,455	2,399	0,055	0,084	IgG_1	1:500	8.0	B
14	***206E3***	3,071	3,001	0,036	0,020	IgG_{2a}	1:500	8.0	A2
15	206H10	2,214	0,525	0,095	0,082	–	–	–	–
16	***206H11***	2,544	2,651	0,024	0,074	IgG_1	1:800	8.0	B
17	207B10	2,731	2,801	0,018	0,045	IgM	–	–	–
18	***207F3***	2,918	2,899	0,042	0,052	IgG_{2b}	1:800	20.0	A2
19	207G4	2,541	2,282	0,028	0,072	–	–	–	–
20	207G8	2,839	2,892	0,048	0,070	IgM	–	–	–
21	208D5	2,246	0,066	0,036	0,099	–	–	–	–

Remarks. [1)] The average values of the results of hybridoma supernatants testing in 4 replications ($p<0.05$). [2)] The parameter is not defined.

Among 21 hybridomas selected during the second screening to further properties were only 16 clones that were characterized by relatively high signal to human IgG and Fc-fragments of IgG and showed no cross-reactivity with other classes of immunoglobulins. At the next phase of work it should be defined criteria for assessing antibodies for further characterization of selected clones of hybridomas. Based on published data and our own experience [1, 4-12] analysis of hybridomas was

carried out for following criteria: McAbs titer in the culture fluid, McAbs affinity and isotyp. Selection of McAbs for the first two criteria (titer and affinity) had to provide high levels of specificity and sensitivity of ELISA using such antibodies.

Studying isotypes of 17 McAbs we obtained the following results: 8 antibodies were IgG_1 isotype, 3 – IgG_{2a} isotype, 3 – IgG_{2b} isotype, 3 – IgM isotype (Fig. 1). The set of obtained data (activity in ELISA, isotype, titer and McAbs affinity constant) used for final selection of hybrid clones for subsequent thawing, cloning, building and accumulation of antibodies. For further studies did not use the hybridomas that produce antibodies of IgM isotype. In addition, the advantage provided clones with high titer ($\geq$ 1:500) and affinity constant ($\geq 8{,}0 \times 10^9$ M^{-1}) and intense signal in the indirect ELISA.

Focusing on the established selection criteria, we formed 4 groups of McAbs which are not promising for developing of highly informative (sensitive and specific) immunoassay methods: 1) McAbs, that are specific to the Fab-fragment of IgG molecule (clones 206H10, 207G4, 208D5); 2) McAbs, which have relatively high signal in the indirect ELISA, but belonged to the IgM-antibodies, that means further non effective approach of isolation and purification of McAbs from ascitic or culture fluid (clones 204H7, 207B10, 207G8); 3) McAbs, which have relatively high signal in the indirect ELISA, but had relatively low titer in culture fluid and/or affinity constant (clones 201C9, 202B11); 4) McAbs, which are showed cross-activity to human IgA and/or IgM by the result of indirect ELISA testing (clone 201A2). Thus, the analysis of data on 19 hybrid clones has led to further studies that were selected 12 hybrids (Table 1): ***201C8***, ***201F10***, ***202B7***, ***202D10***, ***203D10***, ***203G4***, ***206B2***, ***206C8***, ***206C12***, ***206E3***, ***206H11***, ***207F3***.

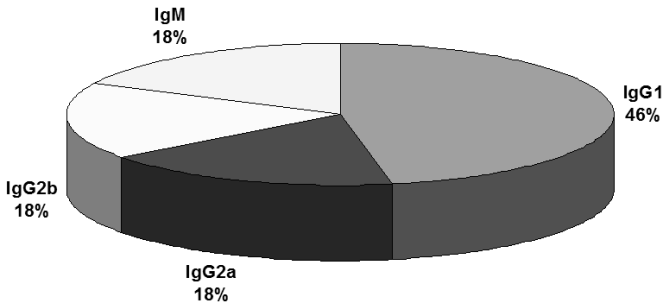

Fig. 1. The distribution of isotypes among obtained monoclonal antibodies

Cloning of hybridomas was carried out by the most simple and effective way (limiting dilution method). All hybridomas were cloned 2-3 times up to complete stability in the level of McAbs synthesis. At the first cloning of hybridomas only 40-60 % of clones gave positive results. The second and third cloning procedure provided high level of positive response (90-100 %). Such developments are likely explained by the gradual stabilization of the total genome of hybrid cells after cryopreservation, including unlocking the genes responsible for the synthesis of immunoglobulins. Isolated positive clones from 96-well plates were transplanted into bigger 24-well plates; hybridomas has been grown, and injected into mice Balb/c for ascites formation. After 7-10 days accumulated ascites were taken out from animals. One mouse gave an average of 10 ml of ascitic fluid. After McAbs isolation of ascites it was used for the synthesis of peroxidase conjugates.

Comparative epitop characteristic of McAbs were performed using a competitive ELISA. Results of competing effects were calculated as the percentage of decrease in activity of McAbs conjugate in the presence of competing antibody. In this embodiment, setting competitive ELISA full or partial decrease in activity is a measure of the total or partial similarity epitop specificity of investigated McAbs. Instead, compared activity with the control McAbs conjugate indicated different competing epitop specificity. Thus, as a result of such testing epitop comparative profile for each monoclonal antibody was obtained (including 11 variants of competition with other McAbs). Correlation analysis was used to facilitate the analysis of such large data of compare profiles. The degree of

similarity was the correlation coefficient profiles provided its statistical significance ($p < 0,05$). High values of positive correlation coefficients between profiles ($0,75 \le r \le 1,00$) were interpreted as evidence of equal epitop specificity, lower values ($0,44 \le r < 0,75$) were considered as an indicator of cross-epitopes and statistically significant coefficients ($r < 0,44$) were regarded as evidence of independent, wholly distinct epitopes.

Table 2 – Comparative epitop characteristic of monoclonal antibodies to human IgG

Epitops	McAbs	Epitop regions and epitops											
		B				**A**							
		B				**A1**			**A2**				
		201F10	**206C12**	**206H11**	**203G4**	**206B2**	**202B7**	**202D10**	**203D10**	**206E3**	**201C8**	**207F3**	**206C8**
B	**201F10**	**1,00**	+	+	+	–	–	–	–	–	–	–	–
	206C12	**0,96**	**1,00**	+	+	–	–	–	–	–	–	–	–
	206H11	**0,91**	**0,94**	**1,00**	+	–	–	–	–	–	–	–	–
	203G4	**0,92**	**0,97**	**0,98**	**1,00**	–	–	–	–	–	–	–	–
A1	**206B2**	0,07	0,12	0,11	0,16	**1,00**	+	+	+	–	+	±	+
	202B7	0,09	0,11	0,09	0,19	**0.90**	**1,00**	+	+	+	±	±	–
	202D10	0.05	0.08	0.04	0.05	**0.94**	**0.98**	**1,00**	+	–	–	–	±
A2	**203D10**	0.27	0.40	0.14	0.09	**1,00**	**0.83**	**0.92**	**1,00**	+	+	+	+
	206E3	0,12	0,30	0,16	0,05	0,40	**0,90**	0,40	**0,94**	**1,00**	±	+	+
	201C8	0,17	0,14	0,16	0,19	**0,87**	*0,52*	0,37	**0,91**	**0,92**	**1,00**	+	+
	207F3	0,19	0,18	0,13	0,13	*0,53*	*0,45*	0,35	**0,82**	**0,95**	**0,81**	**1,00**	+
	206C8	0,25	0,09	0,18	0,30	**0,76**	0,42	*0,61*	**0,95**	**0,97**	**0,98**	**1,00**	**1,00**

Remarks. McAbs has been related to epitop regions and epitopes by its correlation coefficients (r). Statistically significant r ($p < 0,05$) are in bold. The symbol "+" means common epitopes ($0,75 \le r \le 1,00$), "±" – cross epitopes ($0,44 \le r < 0,75$), "–" – independent epitopes ($r < 0,44$).

Comparative epitop characterization of monoclonal antibodies showed that the studied McAbs directed against two epitop regions (ER) of human IgG molecule (conditionally designated by us as A and B epitop regions) (Table 2). 8 monoclonal antibodies referred to epitopnoho region A (ER-A). It should be noted that the reactivity of this group of McAbs is characterized by an average homogeneity, however, EP-A contains 2 McAbs epitopes with high homogeneity: 3 antibodies (206B2, 202B7, 202D10) refers to the epitope A1 (average coefficient is 0,94), and 5 antibodies (203D10, 206E3, 201C8, 207F3, 206C8) refers to the A2 epitope (average coefficient is 0,93). It should be noted that there is a pronounced cross-reactivity between the monoclonal antibodies of epitopes A1 and A2 (average coefficient is 0,62). These data indicate the spatial proximity of epitopes A1 and A2. Another 4 McAbs (201F10, 206C12, 206H11, 203G4), directed to epitope B (average coefficient is 0,95). It should be noted that it is not observed cross-reactivity between the monoclonal antibodies of EP-B and EP-A epitops (correlation coefficients is 0,15). This indicates a quite different spatial localization of epitopes of these two regions.

CONCLUSIONS

1. Set of 12 new monoclonal antibodies to human IgG has been obtained. Following biological properties of obtained McAbs has been studied: activity in the indirect ELISA, specificity within IgG molecule (Fab- or Fc- fragment), cross-reactivity with other classes of serum immunoglobulins (human IgA and IgM), titer in the culture fluid, affinity constant, and relative epitop specificity.

2. Criteria for selecting of monoclonal antibodies for its further usage for the development of highly sensitive and specific immunoassay methods have been founded. McAbs with following characteristics are the most promising for this kind of goals: it has to be directed to the Fc-fragment of IgG, has a high signal in the indirect ELISA, has no cross-reactivity with other classes of immunoglobulins, has titer in the culture fluid not less then 1:500, and affinity constant not less then 8.0×10^9 M^{-1}. McAbs of IgM isotype also are not suitable for effective biotechnological approaches.

3. Further research may focus on the establishment of the serological diagnostic sets (with obtained monoclonal antibodies), based on the determination (detection) of specific IgG- antibodies.

LITERATURE

1. Галкін О.Ю. Моноклональні антитіла до імуноглобулінів людини: особливості одержання та застосування / О.Ю. Галкін, І.В. Ніколаєнко, М.Я. Співак // Імунологія та алергологія. – 2005. – № 1. – С. 3-9.
2. Preparation of highly purified human IgG, IgM, and IgA for immunization and immunoanalysis / [Nikolayenko I.V., Galkin O.Yu., Grabchenko N.I. et al.] // Ukrainica Bioorganica Acta. – 2005. – Vol. 2, №2. – P. 3-11.
3. Galkin O.Yu. Approaches to the synthesis of conjugates for enzyme immunoassay test-systems and evaluation of their use for diagnostics of infectious diseases / O.Yu. Galkin // Український журнал клінічної та лабораторної медицини. – 2010. – Т.5, № 4. – С. 54-60.
4. Принципи, особливості та застосування гібридомної технології / І.В. Ніколаєнко, Л.М. Шинкаренко, О.Ю. Галкін, М.Я. Співак // Імунологія та алергологія. – 2003. – №4. – С. 7-17.
5. Галкін О.Ю. Порівняння схем імунізації мишей лінії Balb/c для одержання моноклональних антитіл до IgM людини / О.Ю. Галкін, О.М. Дуган // Імунологія та алергологія. – 2009. – №1. – С.68-73.
6. Галкін О.Ю. Порівняння схем імунізації мишей лінії Balb/c для одержання моноклональних антитіл до IgA людини / О.Ю. Галкін, О.М. Дуган // Науковий вісник Національного університету біоресурсів та природокористування України. – 2009. – Вип. 134, Ч. 1. – С.88-97.
7. Goding J. Monoclonal Antibodies: Principles and Practice [Monograph] / James Goding. – San Diego: Academic Press, 1996. – 492, [1] p. – (Third Edition).
8. Johnstone A. Immunochemistry 2 : A Practical Approach / A. Johnstone, M. Turner. – Oxford: IRL Press, 1997. – 270, [1] p. – (Practical Approach Series).
9. Evaluation of thirty-one mouse monoclonal antibodies to human IgG epitopes / [Reimer C., Phillips D., Aloisio C. et al.] // Hybridoma. – 1984. – Vol. 3, №3. – P. 263-275.
10. Harlow E. Antibodies. A Laboratory Manual / E. Harlow, D. Lane. – N.-Y.: Cold Spring Harbor. – 1988. – 726, [1] p.
11. Моноклональные антитела к подклассам IgG человека: получение и исследование специфичности / [Климович В.Б., Самойлович М.П., Крутецкая И.Ю. и др.] // Иммунология. – 1998. – №3. – С. 27-31.

12. Панель моноклональних антитіл для внутрішньотипової диференціації поліовірусів II типу / [Широбоков В.П., Ніколаєнко І.В., Копаниця Л.В. та ін.] // Мікробіол. журн. – 1997. – №6. – С. 27-35.

13. Kohler G. Continuous cultures of fused cells secreting antibody of predefined specificity / G. Kohler, C. Milstein // Nature. – 1975. – Vol. 256. – P. 495-497.

14. Lane D. Molecular recognition and the future of monoclonal antibodies / D. Lane, H. Koprowski // Nature. – 1982. – Vol. 296. – P. 200-202.

15. Measurements of the true affinity constant in solution of antigen-antibody complexes by enzyme-linked immunosorbent assay / [Friguet B., Chaffote A., Djavadi-Ohaniance L. et al.] // J. Immunol. Meth. – 1985. – Vol. 77. – P. 305-319.

16. Evaluation of dissociation of antigen-antibody complexes by ELISA / [Kim B., Dikova E., Sheller U. et al.] // J. Immunol. Meth. – 1990. – Vol. 131. – P. 213-222.

17. Бобровник С.А. Влияние полиреактивных иммуноглобулинов сыворотки крови на определение аффинности сывороточных антител / С.А. Бобровник, М.А. Демченко, С.В. Комисаренко // Укр. біохім. журн. – 2010. – 82, №1. – С. 66-69.

18. Tijssen P. Practice and theory of enzyme immunoassays / P. Tijssen // Lab. Techiques in Biochem. and Molecular Biology. – 1985. – Vol. 15. – 674 p.

19. Науково-практичні рекомендації з утримання лабораторних тварин та роботи з ними / Ю. М. Кожем'якін, О. С. Хромов, М. А. Філоненко, Г. А. Сайфетдінова. – К.: Авіцена, 2002. – 156 с.

REFERENCES

1. Galkin O.Ju. Monoklonal'ni antytila do imunoglobuliniv ljudyny: osoblyvosti oderzhannja ta zastosuvannja / O.Ju. Galkin, I.V. Nikolajenko, M.Ja. Spivak // Imunologija ta alergologija. – 2005. – № 1. – S. 3-9.

2. Nikolayenko I.V. Preparation of highly purified human IgG, IgM, and IgA for immunization and immunoanalysis / I.V. Nikolayenko, O.Yu. Galkin, N.I. Grabchenko, M.Ya. Spivak // Ukrainica Bioorganica Acta. – 2005. – Vol. 2, №2. – P. 3-11.

3. Galkin O.Yu. Approaches to the synthesis of conjugates for enzyme immunoassay test-systems and evaluation of their use for diagnostics of infectious diseases / O.Yu. Galkin // Ukrai'ns'kyj zhurnal klinichnoi' ta laboratornoi' medycyny. – 2010. – T.5, №4 – S. 54-60.

4. Pryncypy, osoblyvosti ta zastosuvannja gibrydomnoi' tehnologii' / I.V. Nikolajenko, L.M. Shynkarenko, O.Ju. Galkin, M.Ja. Spivak // Imunologija ta alergologija. – 2003. – №4. – S. 7-17.

5. Galkin O.Ju. Porivnjannja shem imunizacii' myshej linii' Balb/c dlja oderzhannja monoklonal'nyh antytil do IgM ljudyny / O.Ju. Galkin, O.M. Dugan // Imunologija ta alergologija. – 2009. – №1. – S. 68-73.

6. Galkin O.Ju. Porivnjannja shem imunizacii' myshej linii' Balb/c dlja oderzhannja monoklonal'nyh antytil do IgA ljudyny / O.Ju. Galkin, O.M. Dugan // Naukovyj visnyk Nacional'nogo universytetu bioresursiv ta pryrodokorystuvannja Ukrai'ny. – 2009. – Vyp. 134, ch. 1. – S.88-97.

7. Goding J. Monoclonal Antibodies: Principles and Practice [Monograph] / James Goding. – San Diego: Academic Press, 1996. – 492, [1] p. – (Third Edition).

8. Johnstone A. Immunochemistry 2 : A Practical Approach / A. Johnstone, M. Turner. – Oxford: IRL Press, 1997. – 270, [1] p. – (Practical Approach Series).

9. Reimer C. Evaluation of thirty-one mouse monoclonal antibodies to human IgG epitopes / C. Reimer, D. Phillips, C. Aloisio et al. // Hybridoma. – 1984. – Vol. 3, №3. – P. 263-275.

10. Harlow E., Lane D. Antibodies. A Laboratory Manual. – N.-Y.: Cold Spring Harbor. – 1988. – 726 p.

11. Monoklonal'nye antitela k podklassam IgG cheloveka: poluchenie i issledovanie specifichnosti / V.B. Klimovich, M.P. Samojlovich, I.Ju. Kruteckaja i dr. // Immunologija. – 1998. – №3. – S. 27-31.

12. Panel' monoklonal'nyh antytil dlja vnutrishn'otypovoi' dyferenciacii' poliovirusiv II typu / V.P. Shyrobokov, I.V. Nikolajenko, L.V. Kopanycja ta in. // Mikrobiol. zhurn. – 1997. – №6. – S. 27-35.

13. Kohler G. Continuous cultures of fused cells secreting antibody of predefined specificity / G. Kohler, C. Milstein // Nature. – 1975. – Vol. 256. – P. 495-497.

14. Lane D. Molecular recognition and the future of monoclonal antibodies / D. Lane, H. Koprowski // Nature. – 1982. – Vol. 296. – P. 200-202.

15. Measurements of the true affinity constant in solution of antigen-antibody complexes by enzyme-linked immunosorbent assay / B. Friguet, A. Chaffote, L. Djavadi-Ohaniance et al. // J. Immunol. Meth. – 1985. – Vol. 77. – P. 305-319.

16. Evaluation of dissociation of antigen-antibody complexes by ELISA / B. Kim, E. Dikova, U. Sheller et al. // J. Immunol. Meth. – 1990. – Vol. 131. – P. 213-222.

17. Bobrovnik S.A. Vlijanie polireaktivnyh immunoglobulinov syvorotki krovi na opredelenie affinnosti syvorotochnyh antitel / C.A. Bobrovnik, M.A. Demchenko, C.B. Komisarenko // Ukr. biohim. zhurn. – 2010. – 82, №1. – S. 66-69.

18. Tijssen P. Practice and theory of enzyme immunoassays / P. Tijssen // Lab. Techiques in Biochem. and Molecular Biology. – 1985. – Vol. 15. – 674 p.

19. Naukovo-praktychni rekomendacii' z utrymannja laboratornyh tvaryn ta roboty z nymy / Ju. M. Kozhem'jakin, O. S. Hromov, M. A. Filonenko, G. A. Sajfetdinova. – K.: Avicena, 2002. – 156 s.

УДК 796.071:612

ВОЗМОЖНЫЕ ПУТИ КОРРЕКЦИИ АНТИОКСИДАНТНОЙ СИСТЕМЫ ОРГАНИЗМА СПОРТСМЕНОК ВЫСОКОЙ КВАЛИФИКАЦИИ

Голубенко А.В.

Запорожский национальный университет
69600, Украина, Запорожье, ул. Жуковского, 66

020190@ukr.net

Статья посвящена поиску возможных путей коррекции антиоксидантной системы организма спортсменок высокой квалификации. Актуальность исследования антиоксидантной системы защиты организма спортсмена, который регулярно поддается нагрузке высокой интенсивности, не вызывает сомнений и обусловлена необходимостью контроля данных показателей, величины которых в значительной степени влияют на работоспособность спортсмена высокого класса. В нашем исследовании приняли участие 15 девушек 18-20 лет сборной команды аэробики ЗНУ, имеющих спортивный разряд не ниже кандидата в мастера спорта. Предварительное определение биохимических показателей, характеризующих функционирование антиоксидантной системы спортсменок, на начальном этапе исследования показало отсутствие достоверных различий. В экспериментальной группе, спортсменки которой принимали экдистерон, удалось зафиксировать достоверное ингибирование синтеза оксида азота индуцибельной NOS, снижение потребления аргинина аргиназой, повышение синтеза NO конститутивной NOS, понижение уровня мочевой кислоты, и как следствие, ингибирование ксантиоксидазной реакции, которая является причиной возникновения супероксиданион радикала.

Ключевые слова: антиоксидантная система, экдистерон, оксид азота, спортсменки 18-20 лет.

МОЖЛИВІ ШЛЯХИ КОРЕКЦІЇ АНТИОКСИДАНТНОЇ СИСТЕМИ ОРГАНІЗМУ СПОРТСМЕНОК ВИСОКОЇ КВАЛІФІКАЦІЇ

Голубенко А.В.

Запорізький національний університет
69600, Україна, Запоріжжя, вул. Жуковського, 66

020190@ukr.net

Стаття присвячена пошуку можливих шляхів корекції антиоксидантної системи організму спортсменок високої кваліфікації. Актуальність дослідження антиоксидантної системи захисту організму спортсмена, який регулярно піддається навантаженню високої інтенсивності, не викликає сумнівів і обумовлена необхідністю контролю даних показників, величини яких значною мірою впливають на працездатність спортсмена високого класу. У нашому дослідженні взяли