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New Monoclonal Antibodies to Human IgA: Obtaining and Study of Biological Properties

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AG and VS carried out cell engineering experiments and authors AG and AD focused on molecular immunology methods. Author AG conceived and designed the study and wrote the draft of the manuscript. Author LB was a scientific editor of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Preparation of monoclonal antibodies to human IgA, investigation of their properties and selection of the most appropriate McAbs for highly sensitive and specific immunoassay tests.
Methodology: Balb/c mice were used for monoclonal antibodies (McAbs) production. Animals were immunized subcutaneously with purified preparation of human IgA. Immunization duration - 7 days, B cells source - regional lymph nodes. Hybridization of immunocompetent cells and myeloma cells was performed with polyethylene glycol as a fusogen. Screening and subsequent hybridoma clones selection was performed by ELISA-test using human IgA and IgA Fc-fragments, IgG and human IgM. To determine the isotype of McAbs, titer, affinity constants, and to identify its comparative

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epitopic specificity appropriate modifications of ELISA-test were used.

Results: In our experiments new hybrid clones selection scheme to define the most appropriate McAbs for highly sensitive and specific immunoassay tests was developed. It was established that the most prospective were hybridoma clones producing antibodies against Fc-region of IgA molecule. It was proposed to have a comprehensive description of antibodies, which included the establishment of its isotype, titer, and affinity constants. In view of the further use of obtained McAbs for development of highly informative immunoassay methods, only high titer and affinity antibodies were selected. Since IgM-antibodies are characterized by low specificity and affinity, McAbs of such isotype have not been chosen for further study. Focusing on more efficient McAbs purification on protein A based sorbents, antibodies with IgG2a, IgG2b, and IgG3 isotypes were selected. It was established that there is a correlation between the antibody titer in the culture fluid and its affinity constant: antibodies with titer more than 1:500 characterized by an affinity constant not less than 10^9 M^{-1}.

Conclusion: A set of 14 new monoclonal antibodies to human IgA has been obtained. Following biological properties of obtained McAbs has been studied: Activity in the indirect ELISA, specificity within IgA molecule (Fab- or Fc- fragment), cross-reactivity with other classes of serum immunoglobulins (human IgG, and IgM), titer in the culture fluid, affinity constant, and relative epitope specificity. Criteria of McAbs selection for their further use in highly sensitive and specific immunoassay methods have been developed. McAbs with following characteristics are the most promising for these purposes: they should be directed to the Fc-fragment of IgA, have a high signal in the indirect ELISA, absence of cross-reactivity with other classes of immunoglobulins, 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 are not suitable for effective biotechnological approaches.

Keywords: Monoclonal antibodies; hybridomas; human IgA; affinity; epitope mapping.

1. INTRODUCTION

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. IgA is one of the main human antibodies classes, which constitutes 14-16% of total immunoglobulins. Most of the serums IgAs are represented by monomeric molecules. Predominantly IgA is contained on mucosal surfaces, in milk, colostrum, mucosal bronchial and gastrointestinal secretions, bile, and urine [1]. Immunoglobulin A is a bifunctional molecule, which in addition to antigen binding performs some more effector functions (e.g., activates complement, bind to cells and others). Structural regions of the IgA molecules, responsible for effector activity, spatially remote from the antigen binding centers and are mainly localised in Fc-fragment. The antibodies isotypes are also caused by antigenic determinants of Fc-fragment [2].

Specific IgAs in human plasma are the markers of pathogens presence in the organism or infection in anamnesis. Therefore, diagnosis of many infections based on the detection of specific immunoglobulin A in human serum.

One of the methods that is widely used in the diagnosis of infectious diseases are enzyme immunoassay (ELISA). This method can be applied for the pathogen antigens determination and detection of antibodies against them. Development and production of ELISA test kits for the diagnosis of these infections is impossible 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 minuses (non-specific binding, cross-reactivity, lack of studies reproducibility, etc.), which significantly limit their use [4]. In this case more prospective reagents are anti-Igs monoclonal antibodies (McAbs). The advantages of McAbs are the exclusive specificity, homogeneity and the possibility of unlimited production. Having armed by the panel of McAbs to human immunoglobulins it is possible to select some clones with high affinity and specificity, which would be suitable for use in highly sensitive and specific immunoassays. It is known that the development of any ELISA test kit demands as wide range of relevant McAbs as possible, because usually from whole McAbs panel only one or several clones provide satisfactory results [3-4].

Among this anti-IgA McAbs are widely used as conjugates with enzymes (horseradish peroxidase, alkaline phosphatase, etc.) in an indirect ELISA to detect specific antibodies to various pathogens, as well as to determine the total IgA level in human serum (plasma) [1,3-5].

The aim of our study was: preparation of monoclonal antibodies to human IgA, investigation of their properties and selection of the most appropriate McAbs for highly sensitive and specific immunoassay tests.

2. MATERIALS AND METHODS

2.1 Immunization of Animals

An immunization scheme for Balb/c mice was chosen according to results of other authors and our previous experiments [5-11]. The most effective immune response has been usually developed following immunization by antigen mixed with Freund's complete adjuvant (FCA) into hind leg pads [12]. The antigen – human IgA used for immunization – was purified in our laboratory. During the course of immunization (7-8 days) each animal received 50-60 μg of human IgA preparation. Two first injections were carried out with FCA, and the last one – without any adjuvant. On the third day after the last antigen injection, lymphocytes were taken from regional lymph nodes and fused with myeloma Sp2/0 cultured cells.

2.2 Obtaining of Hybridomas

The fusion was made using polyethylene glycol 3500-3700 (Sigma, USA) according to the method reported by Kohler & Milstein method [13] modified by Lane & Koprowski [14]. Obtained hybridoma clones were multiplied on peritoneal macrophage feeder cells in a complete growth medium H-Y (Sigma, USA) supplemented with fetal calf serum (Sigma, USA) and HAT medium (Sigma, USA). The cells were cultivated in 96-well plates for tissue cultures (Costar, USA).

The presence of anti-human MAbs in hybridoma growth media was controlled by an indirect ELISA test on the 10^{th} -12^{th} day of cultivation. The optical density values of supernatant fluids from McAbs-positive cultures were 2-3 times higher comparing to the control conjugate. Cells from such positive wells were transplanted into 24-welled plates with peritoneal macrophage feeder cells in the complete growth medium supplemented with the HT medium (Sigma, USA), propagated and frozen. Obtained cells 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 indirect ELISA test with human antibodies – IgA, IgM, and IgG as well as with IgA Fc-fragments. For further analysis, hybridomas synthesizing the most specific and highly affine McAbs with positive results to human IgA and their Fc-fragments and without cross-reactivity to any other Igs classes, were selected. The selected hybridoma clones were then thawed and cloned several times to reach complete stability of antibody synthesis rates. The cloning was carried out using the limiting dilution method; the hybridoma cells were grown on peritoneal macrophage feeder cells in the complete growth medium. Growing hybridoma cells screening was conducted by the indirect ELISA approach evaluating the interactions of McAbs secreted by these cells with a human IgA preparation.

Hybridoma cells with stabile antibody production were propagated and injected to mice, previously primed by pristane (Sigma, USA), for ascites formation. The MAb purification from ascites fluid was carried out by a double precipitation protocol using 18% and 16% Na_2SO_4 (w/v) [10]. Then obtained McAb preparations were used preparation conjugates with horse-radish peroxidase (HRP).

2.3 Indirect ELISA

The coating of human IgA and its Fc-fragments was carried out overnight in 0.05 M carbonate-bicarbonate buffer (pH 9.6) at 4°C, their concentrations being 5 and 2,5 μg/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 with culture fluids 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 then washed (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 sulphuric acid.

The optical density values were read at wave lengths 450/620 nm using a spectrophotometer.

2.4 Competitive ELISA

This ELISA modification was used for antibodies comparative epitope specificity determination. The coating of human IgA was carried out overnight in 0.05 M carbonate-bicarbonate buffer (pH 9.6) at 4°C, their concentrations being 5 µg/ml. For plate washing, PBS-T (pH 7.2-7.4) was used. The plates were incubated with obtained McAbs conjugates and another McAbs (different concentration: starting 0.4 mg/ml, diluting 2-fold) for 1 h (37°C) and then washed. As control we used the difference between conjugate absorbance and absorbance at the competition of the same antibody. Further procedure was performed as in indirect ELISA.

2.5 Evaluation of McAbs Affinity

The McAbs affinity constants were evaluated according to the inhibition method proposed by B. Friguet [15] and modified by B. Kim [16]. Affinity constants were calculated according to S. Bobrovnik recommendations [17]. Immunoglobulin solutions with different IgA concentrations (10^{-9}-10^{-6} mole per litter) were mixed with McAbs-containing culture fluid samples. After incubation (1 h at 37°C) the mixtures were put into 96-well plate wells previously sensitized by a human IgA preparation and the ELISA test was carried out. Control plate wells contained culture fluid samples without the IgA.

2.6 Determination of McAbs Isotype

The isotype determination of the obtained McAbs was carried out using a standard kit for this procedure – ISO-2 (Sigma, USA). The isotyping was conducted by antigen-mediated ELISA. Hybridoma culture fluids were put into the plate wells sensitized by human IgA. 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.

2.7 Synthesis of HRP-containing Conjugates

The McAb conjugation with the horseradish peroxidase (HRP) was carried out according to periodate oxidation method [18] with some modifications. The ratio McAb/enzyme by mass was 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 mM). For the HRP oxidation, the mixture was incubated during 2 h at the ambient temperature. The solution of the oxidized HRP was mixed with an antibody solution, previously dialyzed against the 0.1 M bicarbonate buffer (pH 9.2). This mixture was then applied to a chromatographic column. Then dry Sephadex G-25 (Fluka, Switzerland) was added (1:3, v/v), and the mixture was incubated for 3 h at the room temperature. The conjugate was eluted from the column. Then $NaBH_4$ aqueous solution (5 mg/ml) was added (1/20, v/v). 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 for 60 min. The obtained solution of a McAbs-HRP conjugate was dialyzed against 0.02 M phosphate buffer containing 0.15 M NaCl.

2.8 Statistical Analysis

The obtained data were analyzed by Student t-test. To evaluate the acceptability of the sample size (number of repeated measurements) when calculating ELISA cut-off experimental significance level P, believing it sufficient if the calculated criterion value of the sample does not exceed critical significance level $\alpha = 0.05$ ($P<0.05$). Correlation analysis (according to Brave and Pirsons [19]) was used for antibodies comparative epitope specificity determination.

3. RESULTS AND DISCUSSION

We obtained more than 900 different hybridoma clones. To select primary effective clones, culture fluids of hybridomas interaction with human IgA were determined. The McAbs specificity was assayed with IgA Fc-fragments. The McAbs cross-reactivity was assessed via testing their interactions with human IgG and IgM to reject cross-reacting hybridoma clones. During the primary testing after hybridization specific antibodies against human IgA were detected in all wells of seven plates. In order to identify the most active hybridomas, culture fluids were several times diluted for further screening. This procedure allowed to reduce significantly background signal. In such a way, 38 clones were selected with the highest signals according to the ELISA results. All selected hybridomas

were cryopreserved, while their culture fluids were taken for further investigations. As a result of a repeated testing, high McAbs activities were confirmed in 28 hybridoma clones (Table 1).

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

The analysis of our results demonstrated the following:

15 clones gave high signals in tests with human IgA and human IgA Fc-fragments and demonstrated absence of cross-reactivity with IgM and IgG molecules (clones 131B4, 131C10, 131C11, 132G8, 132G9, 133C4, 133E9, 134C9, 136A9, 136D6, 136E1, 136E12, 137F2, 137F5, 137F12);

13 clones gave somewhat lower signals both in tests with human IgA and its Fc-fragments but also demonstrated absence of cross-reactivity with IgM and IgG (clones 131A9, 131D11, 132B6, 132F12, 133C8, 133D2, 133G12, 134D8, 134E5, 136D5, 137C10, 137D5, 137H10);

10 clones showed higher signals with human IgA comparing to their Fc-fragments or demonstrated their cross-reactivity with human IgM and IgG (clones 131B10, 132A5, 132G4, 133B7, 133F10, 134H11, 136C3, 136D9, 137C11, 137D7).

Among 38 hybridomas during the second screening only 16 clones with relatively high signal to human IgA and Fc-fragments of IgA and absence of cross-reactivity with other classes of immunoglobulins were selected for further investigations. At the next phase of work, criteria were defined for further characterization and selection of hybridomas clones. Based on other author's results and our previous experiments [1,4-12] analysis of hybridomas was carried out for following criteria: McAbs titer in the culture fluid, McAbs affinity and isotypes. 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.

It should be noted that hybridomas 132B6, 132F12, 133G12, and 137C10 has rather low signal in ELISA (Table 1), but were still chosen for further study because these hybrid clones were comparatively small (number of cells in them was approximately 3-5 times lower compared to the other hybrid clones).

The following results were obtained when Isotyping was done for 28 McAbs; 7 antibodies were IgG_{2b} isotype, 15 – IgG_1 isotype, 4 – IgM isotype, 2 – IgG_{2a} isotype (Table 1). The set of obtained data (activity in ELISA, isotype, titer and McAbs affinity constant) was used for final selection of hybrid clones. For further studies we did not use hybridomas producing antibodies of IgM isotype. In addition, the preference was given to clones with high titer (≥ 1:500), affinity constant ($\geq 8.0 \times 10^9$ M^{-1}) and intense signal in the indirect ELISA. Thus, the data of 28 hybrid clones analysis allowed to select 14 hybrids for further studies(Table 1): 131A9, 131D11, 132B6, 132F12, 133E9, 133G12, 134C9, 136A9, 136D5, 136D6, 136E12, 137C10, 137D5, 137H10.

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 procedures provided the high level of positive response (90-100%). Such developments could be 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 have been grown, and injected into mice Balb/c for ascites formation. After 7-10 days, the accumulated ascites were taken out from animals. One mouse gave an average of 10 ml of ascitic fluid. After isolation of McAbs from ascites, it was used for the synthesis of peroxidase conjugates. McAbs cComparative epitope characterisation of the McAbs was conducted by competitive ELISA. Competing effects were calculated as the percent of McAbs conjugate activity decrease in the presence of competing antibody. In such variant of competitive ELISA, full or partial decrease in activity was a marker of the total or partial similarity of McAbs epitopes specificity. And, on the contrary, McAbs conjugate activity at the same level as control indicated on epitope specificity different from competing antibody. Thus, as a result of such testing epitope comparative profile for each monoclonal antibody

was obtained (including 13 variants of competition with other McAbs). Correlation analysis (according to Brave and Pirsons [19]) was used to facilitate the processing of such large data set. As a degree of similarity was used the coefficient of profiles correlation in case of its statistical significance ($p < 0.05$). High values of positive correlation coefficients between profiles ($0.75 \leq r \leq 1.00$) were interpreted as evidence of equal epitope specificity. Lower values ($0.44 \leq r < 0.75$) were considered as an indicator of cross-epitopes. Statistically significant coefficients ($r < 0.44$) were regarded as evidence of independent, wholly distinct epitopes.

Comparative Epitopes analysis showed that the studied McAbs were directed against 3 epitopes of human IgA molecule (Table 2). It should be noted that the reactivity of this group of McAbs was characterized by an average homogeneity, however, epitope region A contains 3 McAbs epitopes with high homogeneity: 3 antibodies (132F12, 134C9, 131A9) refers to the epitope A1 (mean coefficient is 0.96), 5 antibodies (132B6, 131D11, 133E9, 137C10) refers to the A2 epitope (mean coefficient is 0.94), and 7 antibodies (133G12, 136D6, 136E12, 137H10, 136A9, 137D5, 136D5) refers to the A3 epitope (mean coefficient is 0.87).

Table 1. Characterization of McAbs to human IgA

McAbs	Optical density in ELISA [1)]				Isotype	Titer in cultural liquid [1)]	Affinity constant [1)], 10^9 M^{-1}	Epitope specificity
	IgA	IgA Fc-fragments	IgG	IgM				
131A9	2.235	2.022	0.101	0.922	IgG_{2b}	1:500	12.0	A1
131B4	2.588	2.545	0.095	0.851	IgG_{2b}	1:200	–[2)]	–
131C10	2.577	2.502	0.088	0.075	IgG_1	1:200	–	–
131C11	2.901	2.774	0.111	0.134	IgG_1	1:100	–	–
131D11	2.201	1.899	0.087	0.069	IgG_{2b}	1:1000	2.5	A2
132B6	1.780	1.802	0.089	0.092	IgG_{2b}	1:500	2.5	A2
132F12	1.998	2.010	0.078	0.075	IgG_{2b}	1:1000	12.0	A1
132G8	2.886	2.741	0.103	0.099	IgM	–	–	–
132G9	2.732	2.632	0.089	0.095	IgG_1	1:200	–	–
133C4	2.665	2.601	0.112	0.105	IgG_{2b}	1:200	–	–
133C8	2.401	2.125	0.093	0.073	IgG_{2b}	1:200	–	–
133D2	2.011	1.999	0.077	0.082	IgM	–	–	–
133E9	2.685	2.667	0.096	0.083	IgG_{2a}	1:1000	5.0	A2
133G12	1.423	1.586	0.067	0.074	IgG_1	1:1000	10.0	A3
134C9	2.632	2.662	0.097	0.089	IgG_{2b}	1:500	14.0	A1
134D8	2.345	2.220	0.083	0.079	IgM	–	–	–
134E5	2.441	2.247	0.077	0.082	IgG_1	1:100	–	–
136A9	2.562	2.441	0.096	0.094	IgG_{2b}	1:800	10.0	A3
136D5	2.321	2.322	0.093	0.083	IgG_{2b}	1:1000	11.0	A3
136D6	2.952	2.801	0.099	0.091	IgG_{2b}	1:500	8.0	A3
136E1	3.011	2.899	0.121	0.107	IgG_1	1:200	–	–
136E12	3.031	2.885	0.117	0.110	IgG_{2b}	1:500	5.0	A3
137C10	1.920	1.881	0.075	0.069	IgG_1	1:500	5.0	A2
137D5	2.325	2.380	0.082	0.078	IgG_{2b}	1:800	10.0	A3
137F2	2.821	2.809	0.091	0.086	IgG_{2b}	1:200	–	–
137F5	2.785	2.710	0.088	0.075	IgM	–	–	–
137F12	2.902	2.855	0.095	0.091	IgG_{2b}	1:200	–	–
137H10	2.235	1.998	0.088	0.083	IgG_{2a}	1:1000	10.0	A3

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

Table 2. Comparative epitope characteristic of McAbs to human IgA

Epitopes	McAbs	A													
		A1			A2						A3				
		132F12	134C9	131A9	132B6	131D11	133E9	137C10	133G12	136D6	136E12	137H10	136A9	137D5	136D5
	132F12	1.00	+	+	±	±	±	–	±	±	±	±	±	–	+
A1	134C9	0.98	1.00	+	±	+	–	–	±	+	–	±	+	–	+
	131A9	0.95	0.95	1.00	+	±	±	±	±	±	±	±	±	+	±
	132B6	*0.67*	*0.49*	*0.79*	1.00	+	+	+	±	±	+	±	±	–	–
A2	131D11	*0.65*	*0.82*	*0.49*	0.98	1.00	+	+	±	±	+	±	±	±	±
	133E9	*0.59*	0.42	*0.46*	0.89	0.89	1.00	+	±	±	±	–	±	±	±
	137C10	0.39	0.35	*0.68*	0.96	0.91	0.99	1.00	±	±	±	+	±	±	±
	133G12	*0.46*	*0.45*	*0.46*	*0.61*	*0.47*	*0.74*	*0.70*	1.00	+	+	+	+	±	+
	136D6	*0.45*	*0.78*	*0.45*	*0.72*	*0.65*	*0.74*	*0.46*	0.89	1.00	+	+	+	+	+
	136E12	*0.45*	0.13	*0.45*	*0.78*	0.87	*0.46*	*0.54*	0.79	0.85	1.00	+	+	+	+
A3	137H10	*0.46*	*0.47*	*0.47*	*0.46*	*0.65*	0.31	0.77	0.85	0.86	0.94	1.00	+	+	+
	136A9	*0.45*	0.78	*0.46*	*0.48*	*0.55*	*0.62*	*0.63*	0.95	0.95	0.93	0.88	1.00	+	+
	137D5	0.14	0.19	0.77	0.41	*0.58*	*0.62*	*0.71*	*0.47*	0.78	0.98	0.87	0.85	1.00	+
	136D5	0.76	0.76	*0.46*	0.38	*0.65*	*0.70*	*0.74*	0.91	0.91	0.84	0.95	0.96	0.93	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 ≤ r ≤ 1.00), "±" – cross epitopes (0.44 ≤ r <0.75), "–" – independent epitopes (r <0.44)

4. CONCLUSION

A set of 14 new monoclonal antibodies to human IgA has been obtained. Following biological properties of obtained McAbs have been studied: activity in the indirect ELISA, specificity within IgA molecule (Fab- or Fc- fragment), cross-reactivity with other classes of serum immunoglobulins (human IgG, and IgM), titer in the culture fluid, affinity constant, and relative epitope specificity.

Criteria of highly specific monoclonal antibodies selection have been developed. McAbs with following characteristics are the most promising for the development of highly sensitive and specific immunoassay methods:

- they should be directed to the Fc-fragment of IgA,
- have a high signal in the indirect ELISA,
- demonstrate absence of cross-reactivity with other classes of immunoglobulins,
- have titer in the culture fluid not less then 1:500,
- have affinity constant not less then 8.0×10^{9} M^{-1}.

McAbs of IgM isotype are not suitable for effective biotechnological approaches.

It was established that human IgA molecule contained 1 main immunodominant epitope region, which includes three epitopes.

Further research may focus on the development of serological diagnostic sets (with obtained monoclonal antibodies), for the determination (detection) of specific IgA-antibodies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Galkin OY, Nikolayenko IV, Spivak MY. Monoclonal antibodies against human immunoglobulines: Features of obtaining and application. Immunol. Allergol. 2005; 1:3-9. (in Ukrainian).
2. Nikolayenko IV, Galkin OY, Grabchenko NI, Spivak MY. Preparation of highly purified human IgG, IgM, and IgA for immunization and immunoanalysis. Ukrainica Bioorganica Acta. 2005;2(2):3-11.
3. Galkin OY. Approaches to the synthesis of conjugates for enzyme immunoassay test-systems and evaluation of their use for diagnostics of infectious diseases. Ukrainian J. Clin. Labor. Med. 2010;5(4): 54-60. (in Ukrainian).
4. Nikolayenko IV, Shynkarenko LN, Galkin OY, Spivak MY. The principles, characteristics and use of hybrid technologies. Immunol. Allergol. 2003;4:7-17. (in Ukrainian).
5. Galkin OY, Dugan OM. Comparison charts immunization mice Balb/c to produce monoclonal antibodies to human IgM. Immunol. Allergol. 2009;1:68-73. (in Ukrainian).
6. Galkin OY, Dugan OM. Comparison charts immunization mice Balb/c to produce monoclonal antibodies to human IgA. Scientific Bulletin of National University of Life and Environmental Sciences of Ukraine. 2009;134(1):88-97. (in Ukrainian).
7. Goding J. Monoclonal antibodies: Principles and practice. San Diego: Academic Press. 1996;492.
8. Johnstone A. Immunochemistry 2: A practical approach. Oxford: IRL Press. 1997;270.
9. Reimer C, Phillips D, Aloisio C. Evaluation of thirty-one mouse monoclonal antibodies to human IgG epitopes. Hybridoma. 1984; 3(3):263-275.
10. Harlow E. Antibodies. A laboratory manual. N.-Y.: Cold Spring Harbor. 1988;726.
11. Klimovich VB, Samoilovich MP, Krutetskaya IY. Monoclonal antibodies to human IgG subclasses: Obtaining and investigation of specificity. Immunol. 1998; 3:27-31. (in Russian).
12. Shirobokov VP, Kopanitsa LV, Nikolaenko IV, Lipatnikova KI, Sologub VK. Monoclonal antibodies used to differentiate between poliovirus types 1 and 3. Microbiol J. 2001;63(6):42-52. (in Russian).
13. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975;256: 495-497.
14. Lane D, Koprowski H. Molecular recognition and the future of monoclonal antibodies. Nature. 1982;296:200-202.

15. Friguet B, Chaffote A, Djavadi-Ohaniance L. Measurements of the true affinity constant in solution of antigen-antibody complexes by enzyme-linked immuno-sorbent assay. J. Immunol. Meth. 1985;77:305-319.
16. Kim B, Dikova E, Sheller U. Evaluation of dissociation of antigen-antibody complexes by ELISA. J. Immunol. Meth. 1990;131: 213-222.
17. Bobrovnik SA, Demchenko MA, Komisarenko SV. Effect of poly reactive serum immunoglobulins to determine the affinity of serum antibodies. Ukrainian Biochem. J. 2010;82(1):66-69. (in Russian).
18. Tijssen P. Practice and theory of enzyme immunoassays. Lab. Techiques in Biochem. and Molecular Biology. 1985;15: 674.
19. Guseva EN. Probability theory and mathematical statistics. Moscow: Flinta. 2011;220. (In Russian).

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