

**BIOTECHNOLOGICAL METHOD OF IMMUNE REAGENTS DEVELOPMENT  
ON THE BASIS OF ANTI-IDIOTYPIC ANTIBODIES**

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**Abstract**

*The studying of anti-idiotypic antibodies and their Fab-fragments properties as the basis for the development of biologically safe, effective immunological preparations is the aim of research. Several strains of hybridomas cells producing a monoclonal anti-idiotypic antibodies to Brucella antigen were obtained. Investigations by ELISA and immuno-diffusion reactions showed that in a population of monoclonal anti-idiotypic antibodies there are At2 $\beta$  antibodies that can be used as a "prototype" Brucella antigen. It is planned to study diagnostic properties of anti-idiotypic antibodies and their Fab - fragments with the purpose of making of Brucella antigen immunochemical analogs.*

**Key words:** *anti-idiotypic antibodies, Fab-fragments, immunological preparations, hybridomas cells, monoclonal anti-idiotypic antibodies, o Brucella antigen*

**INTRODUCTION**

The studying of the production process of anti-idiotypic antibodies, their function in processes of immune system functioning regulation is actual direction of modern immunology. Possibilities to use anti-idiotypic antibodies as immunochemical alternatives of antigens are studied very actively over the last years for the development of protective and diagnostic means. The most optimal is use of biotechnological methods, in particular, monoclonal antibody technology. Monoclonal antibodies have been widely spread as the unique and effective instrument in immunological researches.

Antibody synthesis against proper idiotypes was firstly demonstrated by S. Rodkey (1974). He separated and cleaned trimethylammonium hapten antibodies from one rabbit. Injection of these antibodies to the same rabbit one year later stimulated synthesis of anti-idiotypic antibodies.

A.A. Kush, T.A. Possevaya, V.I. Simonov and others (1991) received murine anti-idiotypic antibodies against immunoglobulin of rabbit directed to herpes simplex virus of type 1 (HSV1). Received anti-idiotypic antibodies neutralized infectious activity of virus. S.V. Spitsyn, E.I. Drobyshevskaya, O.I. Barkhatova and others studied the possibility to receive syngeneic anti-idiotypic antibodies to monoclonal antibodies against Legionella pneumophila and possibility to use them for stimulation of immune response to this microorganism. Authors in syngeneic system obtained a hybrid cell line synthesizing monoclonal anti-idiotypic antibodies to monoclonal antibodies against cytolysin Legionella pneumophila. Obtained anti-idiotypic antibodies stimulated antibody generation of BALB/c mice which part reacted with initial antigen.

Anti-idiotypic antibodies were successfully used for virus research, identification of cell receptor of reovirus, polyomavirus, measles virus, human cytomegalovirus, bovine viral diarrhea virus, rhinovirus and tick-borne encephalitis virus (E.V. Protopopova, A.D. Khussainov and others, 1996).

There is information in literature on the obtaining and perspective use of anti-idiotypic antibodies to haptens (T.Yu. Mareeva, 1995).

G.P. Yermakov, S.V. Spitsyn, V.G. Nesterenko studied idiotypic immunogenicity of monoclonal anti-idiotypic antibodies in syngeneic system (1993).

Research findings of many authors prove the perspective of anti-idiotypic antibody use as diagnostic and preventive preparations. Thereby, V.A. Fedorova, Z.L. Devdariani received murine anti-idiotypic antibodies to Y. Pestis proteins encoded by virulence plasmid of plague agent and able to protect 77-80% of mice against experimental plague (2006).

B.B. Gnedenko, S.G. Morozov, I.E. Gribova, T.P. Klyushnik and others used Fab-fragments of anti-idiotypic antibodies upon the development of modified immune-enzyme test system for studying of shift-like schizophrenia (2006).

Researches of last years demonstrated the perspective of anti-idiotypic antibody property study. So, Perosa Federico, Favoino Elvira, C. M. Antonietta inform that murine anti-idiotypic monoclonal antibodies 16D7 identify paratope of monoclonal antibodies HP2/6 against synthetic human molecules CD4. Studying of individual H and L chains to the ability to suppress binding of monoclonal antibodies with antigen CD4 or monoclonal antibody 2 demonstrated that only L chains of monoclonal antibody 16D7 specifically suppress these interactions (2006).

Scientists from the Chinese Agrarian University studied the effectiveness of anti-idiotypic antibodies against Marek's disease virus and reproductive and respiratory syndrome virus (2005).

Polyclonal anti-idiotypic antibodies were received in Kazakhstan and used for early life-time diagnostics of botfly diseases of horses (Zh.U. Yespanov, 1994).

Also there is information on use of polyclonal anti-idiotypic antibodies upon studying of blood protozoan diseases of animals (G.S. Shabdarbayeva, 2006).

Thereby, literature review demonstrates the wide application of anti-idiotypic antibodies in clinical and biological researches both in medicine and veterinary.

According to data of scientific literature review, the most part of anti-idiotypic antibodies were received to viral antigens. Monoclonal antibodies specific to the initial antigens are used for the obtaining of anti-idiotypic antibodies. We also in our researches used monoclonal antibodies obtained to polysaccharide antigen of Brucellas.

## METHODS OF RESEARCH

BALB/c mice were immunized with pure preparations of monoclonal antibodies (MCA) specific to polysaccharide antigen of Brucellas: 4 times with 2-week interval, in concentration 100 mkg with full Freund's adjuvant. 150 mkg of MCA in phosphate-buffered saline were injected in one week after the last immunization. Research was made in the seventh day after the last immunization of serum by means of enzyme-linked immunoassay (ELISA) with biotin-modified MCA. Murine splenocytes which serum contained a high titre of anti-idiotypic antibodies hybridized with myeloma cells X63-Ag8-563 according to generally-recognized method of monoclonal antibody technology. At the 7-10<sup>th</sup> days after hybridization, cultural liquid was tested to the presence of anti-idiotypic antibodies in reaction with biotin-modified monoclonal antibodies used for immunization of mice by ELISA. Elaboration of preparative number of MCA was made by cultivation of hybrid cells *in vivo*. Purification of anti-idiotypic antibodies was made by desalting using anion-exchange micro-centrifugal column.

Anti-idiotypic antibodies At2 $\beta$ , being the “internal image” of polysaccharide brucellar antigens were identified by immunodiffusion reaction and as follows:

Method 1. Identification of anti-idiotypic antibody ability to react with xenogenic sera positive to brucellosis.

Polystyrene plates were sensitized by xenogenic monospecific sera positive to brucellosis, in concentration 5 mkg/ml in bicarbonate buffer pH 9.5. Blocking was made by 1% solution of bovine serum albumin (BSA) during 1 hour. After cleaning, serial dilutions of anti-idiotypic antibodies on phosphate-buffered saline with addition of 0.05% twin solution were layered and treated with anti-species conjugates. Reaction was caused by orthophenylenediamine. Normal serum of animals was used as a negative control.

Method 2. Identification of anti-idiotypic antibody ability to induce immune response to the initial polysaccharide antigen of Brucella in organism of test animals.

BALB/c mice were immunized with anti-idiotypic antibodies in number of 100 mkg per one animal; 4 times with two-week interval. At the 4<sup>th</sup> day after the last injection, the immune serum was tested by ELISA to reaction with Brucella antigen. Serum obtained upon immunization by polysaccharide antigen was used as a positive control. Normal serum of mice was used as a negative control.

## RESEARCH RESULTS

Samples of experimental sera were obtained in the process of linear mice immunization by purified preparations of monoclonal antibodies specific to polysaccharide Brucella antigen. Research of the latter by ELISA demonstrated the presence of anti-idiotypic antibodies. Test results of tested sera are shown in the table below.

Table 1 - Test results of immune serum by ELISA

| Serum                | Optical density of serum dilutions |       |       |       |        |        |
|----------------------|------------------------------------|-------|-------|-------|--------|--------|
|                      | 1:100                              | 1:200 | 1:400 | 1:800 | 1:1600 | 1:3200 |
| immunized animal     | 1.795                              | 1.149 | 1.025 | 0.715 | 0.669  | 0.250  |
| non-immunized animal | 0.142                              | 0.058 | 0.039 | 0.033 | 0.034  | 0.023  |

All sera samples tested by ELISA with polyclonal antibodies of rabbits and livestock were titrated to the dilution 1:3200 as evidenced by extinction ratio – 0.250, exceeding negative control ratio – 0.142. After hybridization of mice splenocytes, which sera demonstrated the highest titer, with myeloma cells, growth of hybrid cells was observed at the 7<sup>th</sup> day in 61 holes of seeded plates. The initial testing to anti-idiotypic antibody production was made at the 12<sup>th</sup> day after fusion. Cultural liquid of 39 clones was selected for this research. Production of anti-idiotypic antibodies was observed in supernatant of 9 hybrid cells – 2A5, 2D11, 3E7, 2D10, 2G9, 3C7, 2D8, 2A6, 2D9. Supernatant of hybrid cells was tested by ELISA to the reaction with Brucella antigen and reaction with mono-specific polyclonal sera positive to brucellosis. It should be said that antigens of all tested clones gave positive reaction in both

cases. Therefore, selection of hybrid cells was made comparatively to antibody activity in tested reactions. Results of ELISA are shown in table 2.

Table 2 – Test results of cultural liquids of hybrid clones by ELISA

| Reagent                                                                                                    | Optical density of clone cultural liquid |       |       |       |       |       |       |       |       |
|------------------------------------------------------------------------------------------------------------|------------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
|                                                                                                            | 2A5                                      | 2A6   | 2D8   | 2D9   | 2D11  | 2 G9  | 3E7   | 3C7   | 2D10  |
| Ag                                                                                                         | 0.179                                    | 0.019 | 0.036 | 0.166 | 0.087 | 0.153 | 0.014 | 0.035 | 0.159 |
| Ab                                                                                                         | 0.478                                    | 0.027 | 0.038 | 0.536 | 0.120 | 0.799 | 0.053 | 0.033 | 0.284 |
| Notes: Ag – Brucella antigen, Ab - anti-brucella antibodies;<br>Optical density of negative control – 0.06 |                                          |       |       |       |       |       |       |       |       |

Results of ELISA demonstrate the diversity of antibody nature produced by hybrid cells. At the same time, significant excess of optical density of anti-idiotypic antibody reaction with mono-specific serum permits to make a conclusion that tested samples contain the original anti-idiotypic antibodies. Activity control of obtained hybrid clones during some passages in culture demonstrated that hybridomas of 2G9 and 2D9 strains are the most stable and active hybridomas selected for further tests. Identification of anti-idiotypic antibody class in immune-diffusion reaction demonstrated that hybridomas produce immunoglobulin IgM – 2D9 and IgG2A – 2G9.

These strains were subject to multiple cryo-preservation. Cultivation on feeding macrophage layer demonstrated stability of biological properties after defrosting. Monolayer of cells formed during 3-5 days (Illustration 2).

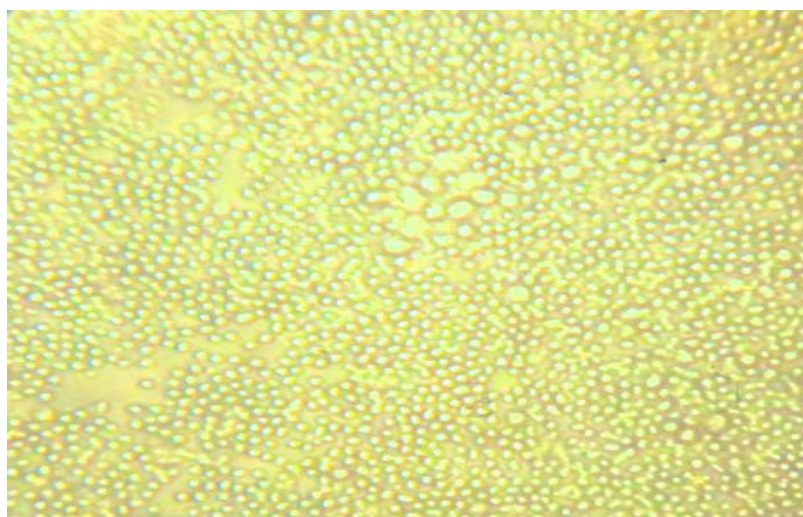


Illustration 2 - Monolayer of hybrid cells in culture

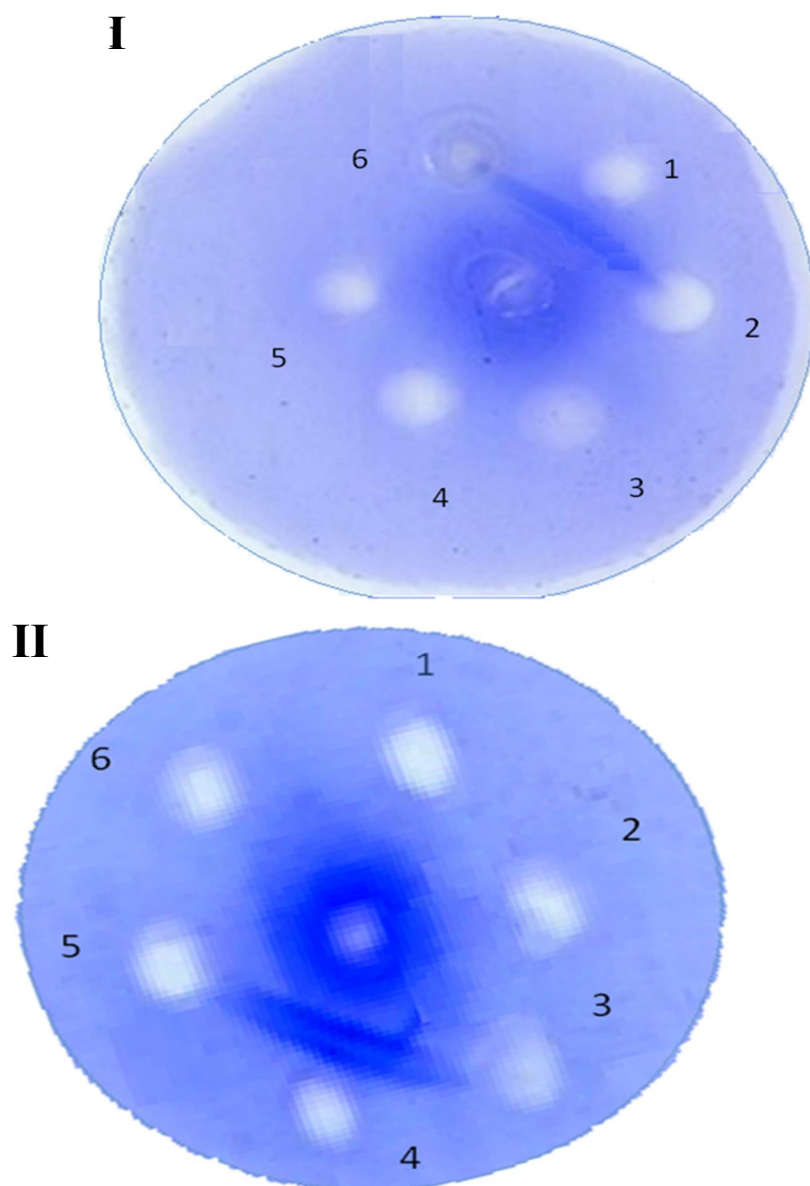


Illustration 1 - Identification of anti-idiotypic antibody class

Notes: I - antibodies of strain 2D9; II – antibodies of strain 2G9

*Immunoglobulin classes: 1- IgM; 2- Ig G1; 3- IgG2A; 4- IgG2B; 5- IgG2C; 6- IgG3*

Strains 2G9 and 2D9 stably produced active monoclonal anti-idiotypic antibodies *in vitro* during some passages.

Cells were cultivated in the medium RPMI-1640 and in accordance with modern methods of cultivation – in serum-free medium EX-CELL where cells were transferred smoothly by reducing number of cow fetus serum by 5%, 2.5% and 0%. Cells of both strains successfully grew in the medium RPMI-1640 and medium EX-CELL. Medium EX-CELL is used for the exclusion of foreign proteins in cultural liquid.

Concentration of monoclonal anti-idiotypic antibodies in ascite after desalting and purification was 2G9 – 8 mg/ml, for 2D9 – 4 mg/ml *in vivo*.

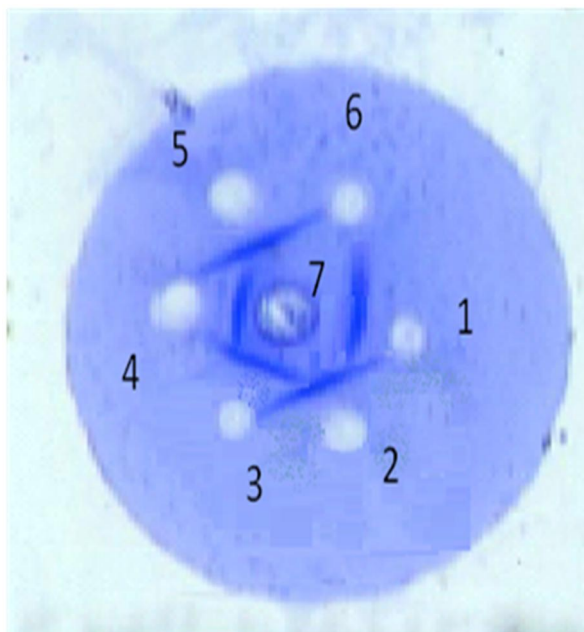
Activity of anti-idiotypic antibodies was tested in indirect version of ELISA. Results are shown in Table 3.

Table 3 – Test results of anti-idiotypic antibody activity produced hybrid strains 2G9 and 2D9 in reaction of indirect ELISA

| Optical density of anti-idiotypic antibodies in ELISA reaction |                                         |       |                                          |       |
|----------------------------------------------------------------|-----------------------------------------|-------|------------------------------------------|-------|
| Dilutions of anti-idiotypic antibodies                         | With rabbit serum against B. melitensis |       | With polyclonal positive livestock serum |       |
|                                                                | 2D9                                     | 2G9   | 2D9                                      | 2G9   |
| 1:100                                                          | 0.631                                   | 0.563 | 0.778                                    | 1.721 |
| 1:200                                                          | 0.462                                   | 0.408 | 0.436                                    | 1.246 |
| 1:400                                                          | 0.277                                   | 0.242 | 0.250                                    | 0.680 |
| 1:800                                                          | 0.187                                   | 0.157 | 0.192                                    | 0.480 |
| 1:1600                                                         | 0.152                                   | 0.111 | 0.101                                    | 0.276 |
| Note – optical density of negative control – 0.172             |                                         |       |                                          |       |

According to values of optical density shown in Table 3, activity of anti-idiotypic antibodies produced by strain 2G9 in reaction with livestock serum was 1:1600, in reaction with rabbit serum – 1:400; anti-idiotypic antibodies of strain 2D9 reacted in titers 1:400 both with livestock serum and rabbit serum.

Since antibodies of strain 2G9 are more active, the further testing was done with this strain. Immunoglobulin of strain 2G9 was tested in reaction of immune-diffusion with various antiserum (Illustration 3).



- 1- rabbit polyclonal antibodies against *Brucella abortus*
- 2- rabbit polyclonal antibodies against *Brucella melitensis*
- 3- livestock serum positive to brucellosis
- 4- small cattle serum positive to brucellosis
- 5- monoclonal antibodies specific to polysaccharide *Brucella* antigen
- 6 – normal serum
- 7- anti-idiotypic monoclonal antibodies of strain 2G9

Illustration 3 – Reaction of immune-diffusion of anti-idiotypic antibodies

According to illustration 3, anti-idiotypic antibodies reacted with all antisera positive to brucellosis and did not react with normal serum of animals that permits to make conclusion on the presence of anti-idiotypic antibodies AT2 $\beta$  which may serve as immunochemical alternative of polysaccharide *Brucella* antigen.

Two immunization schemes: short scheme – during 2 weeks and long scheme – during 2 months were tested for identification of protective properties of monoclonal anti-idiotypic antibodies. Anti-idiotypic antibodies with further injection of buffer dose 150 mkg stimulated synthesis of tertiary antibodies reacted with polysaccharide antigen with antibodies. At expiration of immunization period, tested sera of mice were studied to the presence of tertiary antibodies able to react with polysaccharide and single *Brucella* antigen. Results demonstrated non-effectiveness of short immunization scheme because titers of tertiary antibodies in tested sera were 1:400 in average. After injection of anti-idiotypic antibodies according to long immunization scheme, titers were 1:1600 – 1:3200 respectively.

## CONCLUSION

Research results of anti-idiotypic monoclonal antibody properties produced by hybrid cell strain in reactions of immune-diffusion and indirect ELISA prove the perspective of the selected research and possibility to develop new immune reagents on their basis. At the present time anti-idiotypic antibodies are tested by other versions of ELISA. Researches on the obtaining of their Fab-fragments and methods of their application as immunochemical alternative of polysaccharide Brucella antigen are made at the present time.

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