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Serological Analysis of Immunogenic Properties of Recombinant Meningococcus IgA1 Protease-Based Proteins

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Using the genome sequence of IgA1 protease of *N. meningitidis* of serogroup B, four recombinant proteins of different structure and molecular weight were constructed. These proteins were equal in inducing the formation of specific antibodies to IgA1 protease and had protective properties against meningococci. In the sera of immunized mice, anti-IgA1 protease antibodies were detected by whole-cell ELISA, which indicated the presence of IgA1 protease on the surface of these bacteria. We hypothesized that the protective properties of IgA1 protease-based antigens and IgA1 protease analogs could be realized not only via impairment of bacterium adhesion to the mucosa, but also via suppression of this pathogen in the organism. The presented findings seem promising for using these proteins as the basis for anti-meningococcus vaccine.

Key Words: *IgA1 protease; recombinant proteins; meningococcus; immunogenicity*

IgA1 protease (IgA1pr) is one of the major virulence factors of many pathogens [11-14]; it promotes bacterial colonization of human mucosa and their penetration into internal body medium due to cleavage of secretory immunoglobulins A1 (sIgA1). Therefore, neutralization of IgA1pr at this stage of the invasion would prevent bacterial adhesion on the mucosa and suppress infection development. Mice are a good model for studies of IgA1pr [1], because this enzyme does not cleave mouse IgA1 which allows assessment of immunogenicity and protectivity of enzymatically active preparations. Previous studies [2,3,6,10] have demonstrated that enzymatically active recombinant IgA1pr of serogroup B meningococcus provided pronounced protection (up to 80%) against infection with living virulent culture of meningococci of the basic epidemic serogroups A, B, and C. Our findings allowed us to consider IgA1pr as a promising base for polyvalent vaccine. However, high molecular weight and enzymatic activity of IgA1pr can become serious limitations for vaccine development. It was interesting to assess immunogenicity of IgA1pr-based recombinant proteins with different primary structure and analyze the interaction of antibodies to these antigens with bacterial cell surface.

Here we focused on the search for truncated variants of IgA1pr with different primary structures possessing immunogenic and protective properties for their prospective use as the antigenic base of a polyvalent vaccine.

MATERIALS AND METHODS

We used recombinant proteins I, IIa, IIm, and III prepared, isolated, and purified as described earlier [3]. These peptides were different variants of IgA1 protease of *N. meningitidis* of serogroup B (strain H44/76) that have different primary structure and molecular weight.

Protein I demonstrating high immunogenic and protective activities in animal experiments comparable

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with activity of native IgA1pr isolated from *N. meningitides* culture was used as the reference preparation [2,10].

The study was performed on BALB/c mice weighing 16-18 g (conventional first category mice, Puschino Animal Breeding Center, Moscow Region). The mice were intravenously immunized with the test preparations in a dose of 40 μg or twice with 45-day interval with live virulent meningococcus culture (serogroup B strain H44/76) in a dose of 0.25×10^4 microbial bodies (m.b.) according to the same scheme. The sera were obtained on day 12 after the second immunization.

The antibody levels in the sera of immunized mice were determined using two ELISA variants: using protein I-precoated plate [2] and by whole-cell ELISA after sorption of serogroup B meningococcus strain H44/76 suspension onto the plate [4]. The level of specific antibodies to each antigen was assessed in comparison with the control sera. The serum from mice immunized with protein I or infected with live virulent culture of meningococcus serogroup B in the subletal dose of 0.25×10^4 m.b. according to the above scheme served as the positive control; the serum of non-immunized mice was used as a negative control.

The antibody levels in sera of the immunized mice were also measured by spectral-correlational interference (SCI) [5] with modifications. Sensograms were obtained by successively passing the following solutions through an interferometer (Picoscope15s, Institute of General Physics, Russian Academy of Sciences): *1*) H_2O (mQ); *2*) Tris-M (20 mM Tris, 1.5 M urea, 150 mM NaCl, and 5% sorbitol; pH 7.8); *3*) protein I solution in Tris-M, *4*) Tris-M, *5*) PBS; *6*) BSA solution in PBS (1 mg/ml); *7*) PBS; *8*) a sample of immune or control serum (diluted 1:250 with PBS); *9*) PBS; *10*) conjugate of horse radish peroxidase with goat antibodies to mouse total immunoglobulins in PBS (1:2000 μg/ml); *11*) PBS. Each stage was terminated when the system reached the equilibrium state. The reagents were passed at a rate of 10 μl/min at room temperature. The difference in the thickness of the sorption layers for immune and control sera (Δh) measured at stage 9 was used as the measure of immunogenicity (Fig. 1).

The protective properties of specific antibodies were evaluated by the method of passive defense of intact recipient mice with serum from the immunized donor mice. Intact mice were intravenously injected with 0.2 ml serum from mice immunized with protein I (titer 1:5120 according to ELISA data). The level of protection was evaluated by bacteremia (number of CFU) in the experimental group in comparison with the control. The sera from intact mice served as the control. In 3 h after serum injection, the mice were infected with live virulent meningococcus culture, serogroup B (strain H44/76) and bacteremia was evaluated in 4 h as described earlier [9].

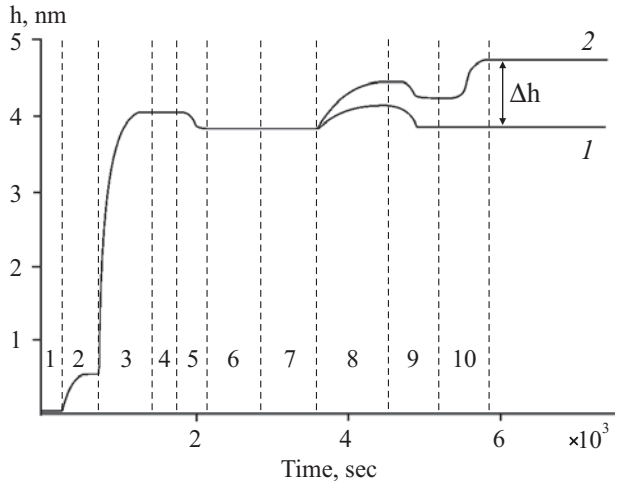

Fig. 1. Sensograms of interaction of components of control serum (*a*) and of serum from immunized animals (*b*).

The results were processed statistically using Microsoft Excel and Probit Analysis softwares using Student's *t* test.

RESULTS

Using recombinant plasmid DNA technology, we created four producer strains with effective expression of recombinant proteins (in the form of inclusion bodies) that represented variants of IgA1pr from *N. meningitidis* serogroup B (strain H44/76) with different primary structures carrying *C*-terminal histidine label (-LEH_6) (Table 1).

Protein I is enzymatically active IgA1pr containing M^1K^2-N^{963} sequence; protein IIa (MA^{28}-N^{963}) also possessing specific enzymatic activity is a variant of protein I lacking signal peptide (M^1K^2-A^{27}); enzymatically inactive protein IIm is a variant of protein IIa with catalytically active serine-267 residue substituted with alanine residue; protein III, a low-molecular-

TABLE 1. Characteristics of IgA1pr Samples in Meningococcus

IgA1pr samples	Protein structure*	Number of a.a.r.	Molecular weight, Da
Protein I	M^1K^2-N^{963}-LEH_6	971	107,596
Protein IIa	MA^{28}-N^{963}-LEH_6	945	104,668
Protein IIm	MA^{28}-N^{963}-LEH_6	945	104,651
Protein III	ME^{135}-H^{328}-LEH_6	203	23,366

Note. Numeration of amino acids residues corresponds to that in precurson protein IgA1pr (http://www.ncbi.nlm.nih.gov/protein/289063650). a.a.r. – amino acid residues.

TABLE 2. Protective Properties of Antibodies to Protein I after Infection with Serogroup B Meningococcus (n=14; $M\pm m$)

Group	Mean number of CFU	t* at $p\leq0.05$
Control (intact mice)	100±12	–
Recipients of immune serum	36±8	3.48
Recipients of intact serum	63±6	2.76

Note. *Student's t test relative to the control.

weight fragment of IgA1pr (ME^{135}-H^{328}), is enzymatically inactive and contains a sequence in the IgA1pr region with high density of potential T- and B-cell epitopes [4,6-8]. Highly purified proteins were prepared from solutions of the corresponding inclusion bodies in urea by affinity chromatography on a Ni-containing adsorbent and after stepwise dialysis were additionally purified on Q-Sepharose [3]. The resultant proteins were used in experiments on animals.

High immunogenic activity of protein I has been demonstrated in animal experiments, as well as its ability to protect the mice against infection with living virulent cultures of three main meningococcus serogroups: A, B and C. The preparation was studied in detail and taken as the base for further development of polyvalent anti-meningococcus vaccine [3]. It was interesting to evaluate the role of antibodies to this protein in protection of animals against meningococcus serogroup B infection. Intact mice were injected with serum from donor mice immunized with protein I or from intact mice. The sera from mice immunized with protein I ensured effective protection from meningococcal infection (~60%) and decreased bacteremia (Table 2).

Since protein I is characterized by the highest molecular weight among the test proteins, the level of specific antibodies to this protein can be used as the reference parameter when studying the protective properties of different IgA1pr variants. Antibodies to protein I in the sera of mice immunized with the studied proteins were detected by two independent methods, ELISA and SCI (Table 3).

Assessment of immunogenic activity of proteins I, IIa, IIm, and III by two independent methods showed that all four preparations demonstrated high activities that did not significantly differed. The antibody titer after immunization with these proteins was significantly higher than after immunization with live microbial cells (natural immunity), which confirmed their high immunogenicity.

Assuming that the protective properties of antibodies could be realized not only upon binding with intact IgA1pr, but also after their direct contact with microbial cells, we studied the ability of anti-protein I antibodies to bind with IgA1pr exposed on meningococcus cell surface (Table 3).

TABLE 3. The Levels of Antibodies in the Sera of Immunized Animals According to ELISA, SCI, and Whole-Cell ELISA

Antigen used for immunization	ELISA*	SCI**	Whole-cell ELISA ***
Protein I	1:3120±512	810±51	1:158±47
Protein IIa	1:2560±146	726±55	1:164±52
Protein IIm	1:2942±206	750±52	1:244±63
Protein III	1:2280±215	706±57	1:147±49
Microbial calls	1:548±141	–	1:1028±301

Note. *Titer of antibodies to Protein I; **thickness of sorption layer, (Δh, nm); ***titer of antibodies to microbial cell. "–", not determined.

The presence of antibodies detected by whole-cell ELISA in the sera of immunized mice attests to the presence of IgA1pr on the surface of *N. meningitides* cells. Our data suggest that adhesion of antibodies on the surface of microbial cells also did not depend on the structure and molecular weight of proteins used for the immunization; therefore, we can speak about their protective role in protection from meningococcal infection.

Thus, the protective properties of IgA1pr-based antigens could be realized not only via impairment of bacterium adhesion to the mucosa, but also via suppression of this pathogen in the body.

The presented findings seem promising for using these proteins as the basis for anti-meningococcus vaccine. Protein III, the preparation with the lowest molecular weight seems to be most promising. The most preferable variant will be used in further studies.

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