Protective Properties of Synthetic Peptides of Outer Meningococcal Membrane

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Peptide fragments of conservative sites of PorA, OpaB, and NspA proteins of the outer membrane of serogroup B meningococci were synthesized. These peptides caused a pronounced protective effect in immunized mice infected with virulent homologous and heterologous strains of serogroups B and A meningococci. The protective effect appreciably increased, if the studied peptides were associated in a polycomponent preparation, which can be used in the construction of meningococcal bivalent B+A vaccine.

Key Words: *meningococcus; protective activity; synthetic peptides*

The absence of effective vaccine for the prevention of meningococcal infection caused by serogroup B *Neisseria meningitidis* (MB) can be explained by specific features of this bacterium. The structure of its capsular polysaccharide is similar to the structure of carbohydrate chains of glycoprotein cells of mammalian peripheral and central nervous system and of some embryonic tissues [7], which makes the vaccine based on this polysaccharide potentially dangerous. The most probable candidates for the vaccine base are outer membrane proteins, but their structure is variable because of mutations developing under the effect of human immune system. Polypeptide sites representing the dominant epitopes of these proteins are mostly liable to mutations.

Phenotypical markers of *N. meningitidis* are presented primarily by class 2/3 proteins, or porins B (PorB), determining the agent serotype, and first-class proteins (PorA) forming the serosubtype [2]. Antibodies to these markers possess protective activity [13]. However, the number of known virulent MB strains circulating in human population and differing by a set of stereotype and serosubtype markers reached several tens and continues to increase due to preparation of new typing monoclonal antibodies [6,8-11]. Therefore, in order to ensure protection from as many as possible circulating agents of MB, the vaccine should include a set of type and serosubtype antigens. However, it was found that if the above-mentioned proteins are present in the same vaccine preparation, their effects are not summed up, but vice versa, they inhibit the immune response to some components of this vaccine [9].

Deciphering of the amino acid sequence of the main proteins of outer MB membrane demonstrated the presence of conservative sites common for the entire Neisseria species [8,11,12], which allowed creation of a vaccine based on synthetic fragments of these proteins. This approach opens the possibility of creating a polyvaccine for the protection from numerous circulating MB strains. A series of peptide fragments consisting of 20-30 amino acid residues, simulating the conservative fragments of MB outer membrane proteins, are immunogenic without conjugation with the carrier protein and protect mice from infection with MB in lethal dose [3-5].

We studied the immunogenic and protective characteristics of some free synthetic peptides of meningococcal surface proteins.

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MATERIALS AND METHODS

Peptides were synthesized by solid-phase method [3-5]. Amino acid sequences of the synthesized peptides corresponding to conservative sites of surface MB proteins are presented in Table 1.

The protective activity of synthetic peptides was evaluated in CBA/La/Sto mice. Group 1 animals (n=7) were immunized with PorA protein peptide 118-143 in a single dose of 100 μg; group 2 mice (n=7) were immunized by MB (strain H44/76) in the sublethal dose of 0.25×10^6 bacterial cells. Group 3 animals (controls, n=7) received complete Freund's adjuvant. MB strains H44/76 (B:15:P1.7,16), B15(B:15:P1.7), 2394(B:2a:P1.2), and MA strain A208 were used in the study. Thirty days after injection the mice of all groups were infected with meningococcus culture (strain H44/76) in doses of 25×10^6, 2.5×10^6, and 0.25×10^6 bacterial cells.

The level of bacteremia in mice served as the indicator of protection from meningococcus; it was evaluated by blood counts of CFU after infection with meningococcus and by the number of mice surviving after infection with meningococcus in the group of animals immunized with peptides in comparison with the control group [1].

TABLE 1. Amino Acid Sequence of Synthetic Peptides

Protein	Amino acid sequence	Peptide*
Por	SGQVKVTKVTKATKAKSRIRTKI	32-51
	ASQAIDPWDSNNDVASQLGIFKRHDD	118-143
	AQLDLSENGDKTKNSTTEIA	273-292
	ISYAHGFDFIERGKKGENTSYDQIIAG	306-332
	SGAWLKRNTGIGNYTQIN	346-363
OpaB	ATGANNTSTVSDYFRNIRTHSI	30-51
	DYFRNIRTHSIHPRVSVGYDPGD	41-63
	DKFDKFKPYIGVRVAYGHVKHQV	131-150
NspA	LRFAVDYTRYKNYKAPSTDFKLY	40-62

Note. *Numbers of amino acids corresponding to their position in the protein are shown.

RESULTS

Blood CFU count in mice immunized with the peptide was 2.5 times lower than in controls (Table 2). In group 2 the level of bacteremia was somewhat higher, which can be due to insufficient concentration of protective antigens injected with this dose of bacteria and by competitive relations of different antigens on the surface of bacterial cell.

Pronounced protection of mice after infection with different doses of meningococcus was observed in animals of groups 1 and 2. The number of survivors was virtually the same in both groups, while in the control group the number of survivors was 3-fold lower (Table 2). These data and a 45.2-52.5 times increase of LD_{50} in groups 1 and 2 in comparison with the control group indicates that peptide 118-143, belonging to highly conservative site of PorA protein, ensured pronounced protection of animals from infection with MB virulent culture comparable to the natural protection forming after this infection.

An obligatory characteristic of the vaccine is animal protection from infection with MB strains differing by serotype and serosubtype markers. Therefore, we evaluated the protective activity of the selected peptides in mice infected with homologous (H44/76) and heterologous (2394, 15) MB strains and with serogroup A meningococcal strain A208. All synthetic peptides used in the study exhibited pronounced protective activity towards all studied meningococcal strains (Table 3), but the intensity of the immune response induced by the studied peptides was different. For instance, PorA peptide 306-332 caused the best protection from the infection with strains H44/76, 2394, and 15, while PorA peptide 118-143 was more effective in infection with strain A208. OpaB peptide 131-150 and NspA 40-62 peptide were more effective in infection with strains A208 and 2394.

These data indicate that B-vaccine should include the most active peptides of all studied outer membrane

TABLE 2. Protection from Meningococcus in Mice Immunized with Peptide and Mice Survived Meningococcal Infection

Group	CFU Number	Death of mice after meningococcus infection in doses (b. c.)[1]			Protection from infection, %	LD_{50}, 10^6 b. c.
		25×10^6	2.5×10^6	0.25×10^6		
1	44 (30-57)*	14/20	5/20	3/20	63	7.87 (6.85-8.87)
2	64 (55-73)**	15/20	6/20	0/20	65	6.78 (5.80-7.78)
3	100 (97-116)	20/20	15/20	11/20	23	0.15 (0.00-1.15)

Note. Range of values is shown in parentheses; [1]number of dead mice/number of infected mice. *p<0.0001, **p<0.005 compared to group 3 (control).

TABLE 3. Bacteremia in Mice Immunized with Peptides after Infection with Meningococcal Strains (*M*±*m*)

Protein	Peptide	CFU count in the blood of mice after infection with meningococcus (strain)			
		H44/76	2394	15	A208
Control		100±7	100±4	100±4	100±6
PorA	118-143	40±3	—	—	5±4
	273-292	65±9	56±8	33±8	51±3
	306-332	32±4	23±12	12±3	22±6
	346-363	62±6	41±4	22±3	7±5
OpaB	30-51	53±6	36±4	29±9	18±8
	131-150	65±7	6±5	—	9±5
NspA	40-62	46±7	12±2	—	9±5

Note. "—": not studied.

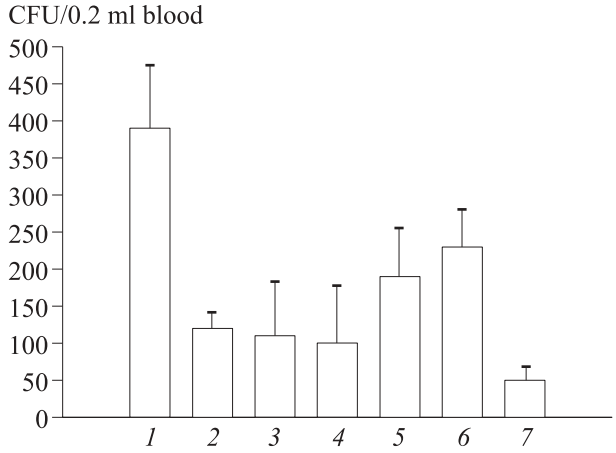

Fig. 1. Protective activity of synthetic peptides and their mixture. *1*) adjuvant; *2*) peptide 30-51; *3*) peptide 131-150; *4*) peptide 306-322; *5*) peptide 346-363; *6*) peptide 40-62; *7*) peptide mixture.

proteins, ensuring the maximum protective effect in infection with different meningococcal strains.

The decreased count of CFU in mice immunized with the peptides was the least pronounced after infection with homologous strain H44/76; the decrease in the content of circulating bacteria was much more pronounced after infection with B strains with heterologous serotype and serosubtype markers and with serogroup A strain A208. As the virulence of meningococcus strains used for infection is different, it seems that less virulent strains are easier eliminated.

Verification of the hypothesis about the efficiency of association of several peptides in one syringe during single immunization of mice (Fig. 1) showed that the number of CFU in the blood of mice immunized with the peptide mixture was much lower than after immunization with individual peptides.

Hence, compounds capable of protecting (individually or in a mixture) mice from not only serogroup B meningococcal strains, but also from serogroup A meningococcus were detected among the synthetic peptides — fragments of conservative sites of meningococcal outer membrane proteins. These data indicate that these peptides can be regarded as potential components of the vaccine from meningococcal infection of different etiology.

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