Mechanisms of Defense Formation against Meningococcal Infection in Mice Immunized with Synthetic Peptides

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Immunization of mice with synthetic peptide fragments of conservative sites of meningococcal outer membrane proteins led to defense formation against infection with virulent serogroup A and B Meningococci. The role of cellular immunity in the formation of defense against meningococcal infection after immunization with the peptides and the possibility of stimulating lymphocyte population with these peptides were demonstrated.

Key Words: *meningococcus; peptides; protection; adoptive transfer*

The absence of available effective vaccines protecting from meningitis caused by serogroup B (MC-B) meningococci (MC) is a result of insufficient knowledge of the mechanisms of immunity formation in this infection. Understanding of cellular mechanisms underlying defense formation against MC and responsible for immunological memory is essential for the creation of effective complex vaccine. The use of synthetic peptide fragments of main MC surface membrane proteins characterized by high protective activity and belonging to conservative MC sites is a promising trend in the creation of new-generation vaccines. The data on the role of cell populations in the response to peptide fragments of MC surface proteins and in the formation of immunological memory are scanty and contradictory [4-8].

We carried out a comparative study of the mechanisms of postinfection and postvaccinal immunity in mice infected with MC-B.

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MATERIALS AND METHODS

The animals were immunized with peptide fragments of MC outer membrane proteins synthesized at Laboratory for Protein Synthesis, M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry [2]. All peptides contained protein sequences exhibiting activity in experimental stimulation of human blood T cells *in vitro* [9].

Protective activity of peptide fragments of MC-B outer membrane proteins was studied on the model of mouse infection with live virulent MC culture [3]. The peptides exhibited pronounced protective activity in experiments on mice with direct infection with live virulent cultures of not only various MC-B strains, but also MC-A and MC-C strains [3].

Donor CBA/La/Sto mice were immunized with synthetic peptide fragments of proteins subcutaneously in a single dose of 100 μg peptide with complete Freund's adjuvant (FA) or twice at 45-day interval, the second immunization with incomplete FA (immune mice). The other group of animals was intraperitoneally immunized with MC-B strain H44/

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76 in a sublethal dose of 0.25×10^6 bacterial cells/mouse (convalescent mice). Control mice received FA according to the same protocol as immune mice.

The role of cellular immunity was studied by the method of adoptive transfer [1]. One month after immunization with the peptide or bacterial infection, lymphocytes were isolated from mouse spleen, thymus, and lymph nodes (inguinal, popliteal, and mesenteric, Fig. 1) in sucrose density gradients (concentrations 20, 40, and 60%). The population of T cells was isolated by negative selection as a result of macrophage adsorption on plastic and removal of B cells (panning) [1]. The content of viable lymphocytes in the suspension was 98%.

In experimental series I, recipient mice intravenously received total lymphocyte suspension in a dose of 20×10^6 cells/mouse. Group 1 mice (n=21) received cell suspension from convalescent mice, group 2 mice (n=30) from immune mice, and group 3 (n=21) mice from controls. After 24 h, the mice were infected with MC-B strain H44/76 in doses of 0.25, 2.5, and 25×10^6 cells. Animal mortality, LD_{50}, and efficiency index (EI; ratio of LD_{50} in the experimental and control groups) were evaluated.

In series II, recipient mice received lymphocytes from convalescents and controls in a concentration of 14×10^6 cells. T cells from convalescents in doses of 7×10^6 and 14×10^6 cells/mouse and from mice immunized with peptides 273-292 and 306-332 in a dose of 7×10^6 cells were transferred. The concentration of 7×10^6 was selected from the ratio of T lymphocytes in injected suspension of total lymphocytes. The mice were then infected similarly as in series I. Four hours after infection, the blood was collected from the retroorbital sinus and inoculated in dishes with solid nutrient medium. The level of bacteremia evaluated by the number of CFU in the blood after infection in comparison with the control served as the index of protection from MC.

In series III, we evaluated the effect of the time of lymphocyte transfer on animal death and CFU level. Experimental mice were injected with lymphocytes from immune mice 24 h before infection and directly during challenge (in a mixture of MC-B strain H44/76; 2.5 and 25×10^6 cells/mouse). Control animals received lymphocytes from control mice. The protective effect was evaluated by the number of CFU in comparison with the control, by the number of survivors on day 5 after infection, and by LD_{50}.

The data were statistically processed using Probit Analysis software.

RESULTS

Lymphocyte population from lymph nodes did not protect mice from infection (EI=1), while splenic and thymic lymphocytes protected mice from MC infection (EI 27.6±3.1 and 7.1±4.3, respectively). Hence, splenic lymphocytes served as the source of immunocompetent cells in studies of the mechanisms of formation of defense against MC infection.

Immunization with peptide 306-332 protected donor mice from MC-B infection, its efficiency was close to the protection in convalescents. Similar results were obtained after transfer of immune lymphocytes to groups 1 and 2 recipient mice (Table 1).

Transfer of lymphocytes simultaneously with bacteria provided better (50%) protection of recipients than transfer of cells 24 h before infection (30% protection, Table 2). These data attest to direct involvement of immune lymphocytes isolated

TABLE 1. Comparison of Protective Effects of Lymphocytes from Immune and Convalescent Donor and Recipient Mice ($M\pm m$)

Group	Mortality after MC infection in a dose of 10^6 bacterial cells			Sum	LD_{50}, $\times10^6$ bacterial cells	EI
	25	2.5	0.25			
Donor mice						
convalescents	15/20*	6/20	0/20	21/60	6.78±1.00*	45
immune	14/20	5/20	3/20	19/60	7.87±1.00*	52
controls	20/20	5/20	11/20	36/60	0.15±1.10	1
Recipient mice						
1	3/7	1/7	0/7	4/21	37.7±1.9+	10.8
2	6/10	4/10	0/10	10/30	17.6±2.2+	5
3	5/7	4/7	1/7	10/21	3.5±1.1	1

Note. Numerator: number of dead mice; denominator: infected mice. $p<0.05$ compared to: *controls; +group 3.

TABLE 2. Relationship between the Time of Lymphocyte Transfer and Protection from MC Infection

Group	Time of lymphocyte transfer	Infective dose, 10^6 bacterial cells		Protection, %
		25	2.5	
Control	24 h before MC-B	10/10	10/10	0
Experimental	Together with MC-B	8/10	2/10	50
	24 h before MC-B	10/10	4/10	30

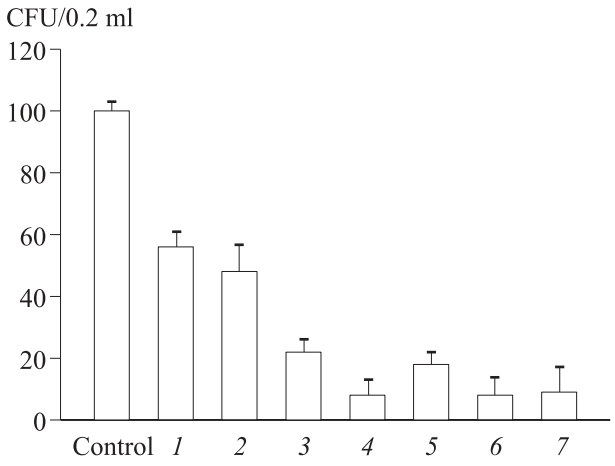

Fig. 1. Protective characteristics of lymphocytes from immune mice against MC-A infection. *1*) peptides 118-143; *2*) 273-292; *3*) 306-332; *4*) 346-363; *5*) 30-51; *6*) 131-150; *7*) 40-62.

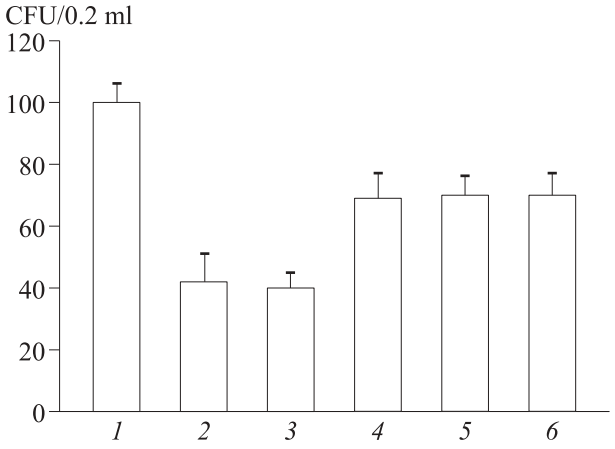

Fig. 2. Protection of recipients mice receiving T cells from MC-B infection. Recipients received 14×10^6 summary splenic lymphocytes from control animals (*1*), 14×10^6 summary splenic lymphocytes from convalescents (*2*), 14×10^6 T-lymphocytes from convalescents (*3*), 7×10^6 T-lymphocytes from convalescents (*4*), 7×10^6 T-lymphocytes from mice immunized with peptide 273-292 (*5*), 7×10^6 T-lymphocytes from mice immunized with peptide 306-332 (*6*).

1 month after immunization into protection of mice from MC infection.

Lymphocytes from donor mice isolated on day 30 after double immunization with peptide fragments of PorA and NspA proteins protected recipient mice also from MC-A strain A208 (Fig. 2). These data suggest to that the selected peptide fragments are highly conservative and can be used for the creation of polyvalent immunity against MC infection of different etiology.

The role of T lymphocytes in protection from MC was evaluated in mice immunized with synthetic peptide fragments 273-292 and 306-332 corresponding to conservative sites of MC PorA protein and in convalescents after MC-B (strain H44/76) infection. Transfer of summary splenic lymphocytes from convalescent donors in a dose of 14×10^6 cells/mouse ensured 58% protection of recipient mice from MC in comparison with the control group (Fig. 2). Transfer of T lymphocytes in this dose provided similarly high protection of recipient mice (60%). Decreasing the concentration by 2 times reduced mouse protection to 30%. The protection of recipient mice after transfer of T lymphocytes from immune mice was also 30%.

These data confirm direct involvement of T cells in the defense from MC infection in mice receiving lymphocytes from convalescents and immune animals.

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