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Peculiarities of the Formation of Antimeningococcus Immunity in Mice Immunized with Fragments of *N. meningitidis* IgA1 Protease

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We studied immunogenicity of two recombinant proteins FR.9 and FR.11-3 created on the basis of fragments of the primary structure of *N. meningitidis* IgA1 protease with different molecular weights containing different sets of T and B epitopes. The proteins actively protect animals infected with live virulent culture of meningococci, serogroups A, B, and C. Analysis of $CD4^+$, $CD8^+$, and $CD19^+$ lymphocyte populations in mouse blood showed predominant contribution of different cell populations to the formation of immune response to different proteins. Injection of FR.11-3 protein to animals did no affect the immunoregulatory index, hence, this protein can be used for creation of immunologically safe vaccine preparation.

Key Words: *IgA1 protease; recombinant proteins; lymphocyte populations*

The problem of creation of a monocomponent polyvaccine for prevention of meningococcal meningitis of different etiology remains unsolved. Many authors suggest that IgA1 proteases (IgA1pr), important factors of bacterial virulence, can be used for protection from these pathogens [8,9]. We have shown in animal experiments that recombinant IgA1pr in active and mutant forms and some short analogs of meningococcal IgA1pr are characterized by high immunogenic and protective activities and protect mice from infection with live virulent culture of meningococci of the main epidemic serogroups (A, B, and C); they also exhibit capacity typical of proteins to the formation of immunological memory [2,4,12].

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The mechanism of protection against meningococcal infection remains not quite clear; the mechanisms of protection of animals immunized with truncated IgA1pr analogs are also unknown. Research of this problem is essential for the choice of optimal structure of the main component of prospective vaccine.

We studied the mechanisms of protection from meningococcal infection in mice immunized with IgA1pr fragments.

MATERIALS AND METHODS

Recombinant proteins, fragments of *N. meningitidis* (serogroup B, strain H44/76) recombinant IgA1pr with different molecular weights: 23 kDa (FR.9) and 59 kDa (FR.11-3) were used in the study [1]. Protein FR.9 is a short analog of protein FR.11-3.

The study was carried out on BALB/c mice (16-18 g). Experimental groups received FR.9 or FR.11-3. Control group mice were not immunized. Each group consisted of 7 mice.

On day 30 after a single and on day 10 after double immunization, serum titers of antibodies to IgA1pr were measured by adsorption of IgA1pr on the plates. The protective activities of the preparations were evaluated on day 12 after double immunization as described previously [2].

The population composition of blood lymphocytes was studied by flow cytometry [3,11]. Blood specimens (200 μl) were collected on day 12 after double immunization. The levels of $CD4^+$ T helpers, $CD8^+$ cytotoxic T lymphocytes, and $CD19^+$ B lymphocytes were measured using monoclonal antibodies CD4 (FITC), CD8 (FITC), and CD19 (PE) (BioLegend) on a FACScan flow cytofluorimeter (BD Biosciences). The data were processed using Flowing Software 2.5.1.

The results of flow cytometry were presented as the percentage of cells of each studied cell population in the total pool of lymphocytes.

The data were processed using Probit Analysis software.

RESULTS

On day 30 after a single immunization of mice by recombinant proteins FR.11-3 and FR.9, the titers of antibodies to IgA1pr measured by ELISA were similar (Table 1); however, on day 10 after double immunization, the titer of antibodies in response to immunization with FR.11-3 was by one order of magnitude higher. It should be noted that despite higher titer of antibodies in mice immunized with FR.11-3, the count of CFU (*i.e*. the number of circulating bacteria in these animals) was significantly higher ($p \leq 0.05$) than after immunization with FR.9 (Table 1). Hence, comparison of the protective activities of the studied proteins and their capacity to antibody production showed no direct correlation between the level of antibodies to IgA1pr and protection of mice from meningococcal infection after immunization with these antigens.

The weaker protection of mice immunized with FR.11-3 despite 10-fold higher titers than after immunization with FR-9 can be explained by differences in the affinity of the forming antibodies. Another possible cause is the difference in the mechanisms of protection induced by these proteins related to cellular immunity.

The results of flow cytometry on day 12 after double immunization showed that immunization of mice with FR.9 and FR.11-3 proteins led to activation of $CD4^+$ and $CD8^+$ T-cell subpopulations (Fig. 1).

The count of $CD4^+$ lymphocytes after immunization with FR.9 protein differed just negligibly from the parameter in the control. Immunization with FR.11-3 protein caused activation of these cells: their count was significantly higher ($p<0.05$) than in the control and after immunization with FR.9 protein. The count of cytotoxic T cells ($CD8^+$) increased significantly in response to FR.9 immunization, but remained within the normal range in response to FR.11-3 immunization. Hence, the population of cytotoxic T cells ($CD8^+$) predominated in the blood of mice immunized with FR.9 by the moment of infection with live meningococcal culture. This fact could be responsible for significant decrease of bacteremia level in these animals with rather low level of specific antibodies to IgA1pr (Table 1). The important role of cytotoxic T cells (T killers) in the mechanism of antibacterial immunity was discussed previously [5,7].

According to published data, the level of circulating antibodies and hence, antibody-producing B lymphocytes ($CD19^+$) play an important role in protection from bacterial infections. It is known that production of antibodies to thymus-dependent antigens is regulated by T helpers ($CD4^+$), their count reaching the peak on day 7 after contact with the antigen and persisting during several days [6,10]. In our experiment, the count of T-helpers measured on day 12 after immunization with FR.11-3 (at the peak of antibody production) was significantly higher than in controls (Fig. 1). The count of antibody-producing lymphocytes ($CD19^+$) in these animals was lower than in the control. By this moment, B-cell populations are presumably transformed into memory cells and redistributed from the blood to

TABLE 1. Immunogenic and Protective Characteristics of Recombinant Proteins FR.9 and FR.11-3

Antigen	Titer of antibodies to IgA1pr		CFU, %		
	day 30	day 10	A	B	C
Control	—	—	100	100	100
FR.11-3 (59 kDa)	1:80	1:10 240	43	38	39
FR.9 (23 kDa)	1:160	1:1280	28	28	23

Note. Day 30: day 30 after a single immunization; Day 10: day 10 after double immunization; CFU was expressed in percent of control, taken for 100%.

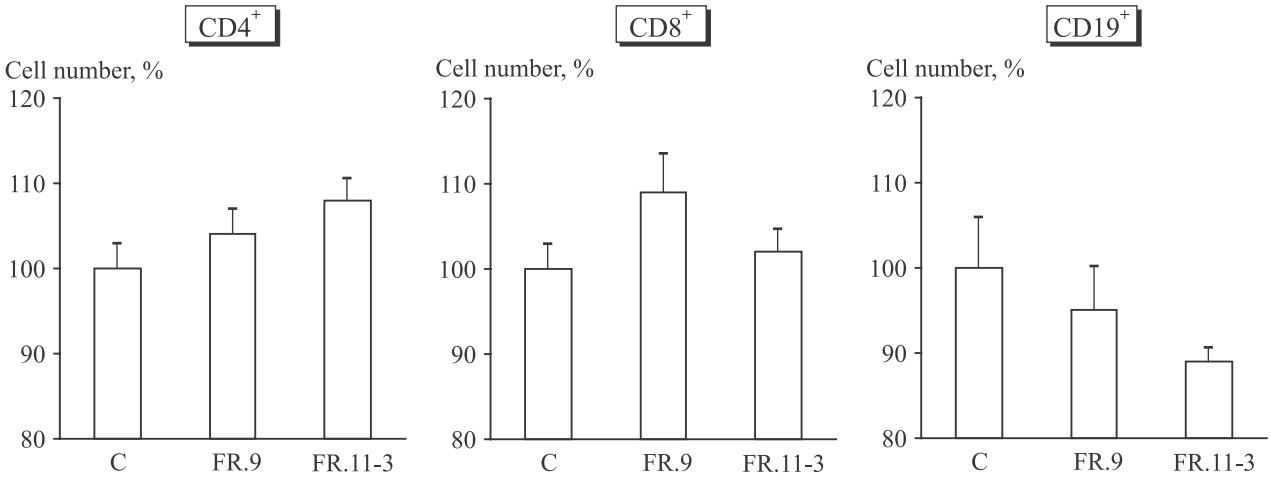

Fig. 1. Induction of T cells ($CD4^+$, $CD8^+$) and B cells ($CD19^+$) in the blood of mice immunized with FR.9 and FR.11-3 proteins on day 12 after double immunization. C — control.

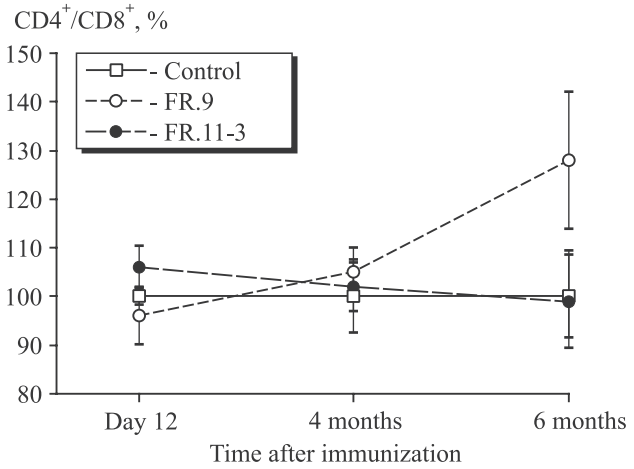

Fig. 2. Immunoregulatory index in mice after double immunization with FR.9 and FR.11-3 proteins.

other organs. Hence, the protection of mice immunized with FR.11-3 protein seemed to be realized mainly by circulating antibodies, previously produced by B cells.

Evaluation of the immune status of mice throughout the entire period of observation after immunization with FR.9 and FR.11-3 proteins (6 months) showed negligible changes in the immunoregulatory index ($CD4^+/CD8^+$) on day 12 after double immunization (Fig. 2). In 4 months, the $CD4^+/CD8^+$ proportion in immunized mice did not differ from the control values. However, in 6 months this parameter sharply increased and differed significantly from the control. The $CD4^+/CD8^+$ proportion remained normal in the group of animals immunized with FR.11-3 protein.

Hence, recombinant protein FR.11-3 caused virtually no changes in the subpopulation composition of T ($CD4^+$, $CD8^+$) and B cells ($CD19^+$) and did not disturb the immunoregulatory balance in mice in the delayed period after immunization.

Our results indicate the possibility of creating immunologically safe drugs with high immunogenic and protective activities on the basis of meningococcal IgA1pr fragments. These proteins seem to be most promising for designing the meningococcal polyvaccine.

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