IMMUNE RESPONSE OF MICE OF DIFFERENT LINES TO MENINGOCOCCAL POLYSACCHARIDE GROUP A ANTIGEN

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Interest in the study of genetic control of the immune response to bacterial polysaccharides is largely due to the unique character of the regulatory mechanisms that control the response of immunocompetent cells to these antigens. The genetic basis of the immune response to pneumococcal polysaccharide antigen has been studied most completely at the present time [4, 7]. There is evidence of the role of genetic factors in the control of the immune response to typhoid Vi-antigen [3]. However, genetic aspects of immunogenesis following injection of meningococcal polysaccharide antigens have not yet been studied.

The aim of this investigation was to determine the level of the immune response to meningococcal polysaccharide group A antigen (MPAA) in mice of different lines and also to examine the role of antigenic mimicry as one possible mechanism of the interlinear differences revealed in the course of the work.

EXPERIMENTAL METHOD

Male mice of lines BALB/cSto, CC57BR/MvSto, CBA/CaLacSto, and C57BL/6JSto and (CBA × C57-BL/6)F_1 hybrids (F_1) aged 2-3 months were used. Meningococcal polysaccharide **group A vaccine** produced by the G. N. Gabrichevskii Moscow Research Institute of Epidemiology and Microbiology [1] was used as the antigen. Mice were immunized intravenously in doses from 0.01 to 2.5 μg and the number of antibody-forming cells (AFC) in the spleen was determined on the 4th-7th day by the method of local hemolysis in gel, using sheep's red blood cells loaded with MPAA for this purpose [2]. In experiments to study absorption, serum from volunteers inoculated with meningococcal polysaccharide group A vaccine were used. Antibody titers were determined in the passive hemagglutination test. The serum was absorbed with a solid residue of spleen cells (SC) containing 45×10^7 cells, to which 400 μl of the test serum was added in different dilutions. The residue was resuspended and incubated for 15 min at 37°C, then again for 30 min at room temperature. The cells were sedimented by centrifugation and the serum transferred to the next tube containing a similar number of SC. For statistical analysis of the results geometric mean values of the number of AFC were used. Confidence intervals were calculated by the formula $M_g \pm tm$ at the $P \leqslant 0.05$ level of significance.

EXPERIMENTAL RESULTS

In the experiments of series I the immune response of mice of different lines to injection of 0.5 μg of MPAA was studied. The number of AFC was determined on the 5th day after immunization. The experiments showed (Fig. 1) that the number of AFC detectable in the spleen of C57BL/6 mice was much smaller thaa in mice of the other lines tested ($P < 0.05$). The F_1 mice occupied an intermediate position between the parental lines for magnitude of the immune response to MPAA. Thus only C57BL/6 mice gave a low level of response to MPAA. The interlinear differences which were discovered were still present when the number of AFC was calculated per 10^7 SC. In C57BL/6 mice (n = 30), for instance, this number was 21 (13-24), whereas in CC57BR mice (n = 21) it was 111 (74-166).

In the experiments of series II the magnitude of interlinear differences in the immune response was studied in C57BL/6 and CC57BR mice at different times after immunization, when

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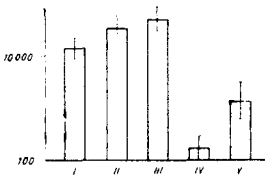

Fig. 1. Immune response of different lines of mice to MPAA. Ordinate, number of AFC per spleen. I) BALB/c, II) CC57BR, III) CBA, IV) C57BL/6, V) (CBA × C57BL/c)F_1 mice.

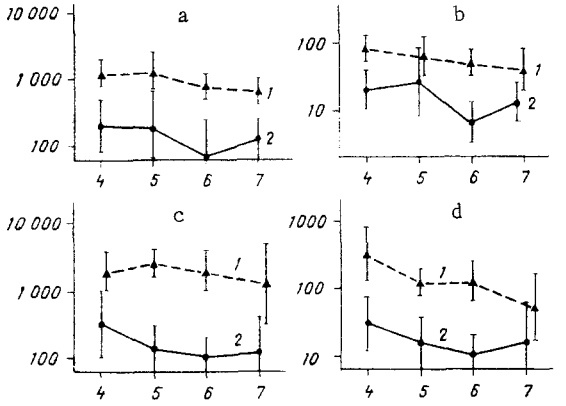

Fig. 2. Effect of dose of MPAA and time after immunization on intensity of immune response of mice. a,b) Immunization with MPAA in dose of 0.05 μg; c,d) immunization with MPAA in a dose of 0.5 μg. Abscissa, days after immunization; ordinate, number of AFC per spleen (a, c) and per 10^7 SC (b, d). 1) CC57BR; 2) C57BL/6.

two doses of antigen were used: 0.05 and 0.5 μg. It will be clear from Fig. 2 that the intensity of the immune response of the two lines of mice did not depend significantly on the time elapsing after injection of the antigen. When a dose of 0.5 μg was used, interlinear differences were discovered on the 4th, 5th, and 6th days after immunization when the number of AFC was counted both in the whole spleen and per 10^7 cells (Fig. 2).

To verify the conclusion that C57BL/6 mice are a line which gives a truly low level of response, the range of doses of MPAA from 0.01 to 2.5 μg was used. The results in Table 1 show that the level of the immune response to all doses of MPAA used remained low. No statistically significant differences could be found between the groups.

One possible explanation of the low immune response of the C57BL/6 mice to MPAA is the hypothesis that mice of this line have natural antigens which cross-react with MPAA, as has been shown for ferritin [5] and *Salmonella typhimurium* [8]. To test this hypothesis serum containing antibodies against MPAA was absorbed four times with SC from mice of different lines. As Table 2 shows, this resulted in some decrease in the antibody titers, but the decrease was the same after absorption by SC of lines giving both a high and a low response. These results cannot be regarded as evidence of the presence of antigenic mimicry, and they are most probably connected with nonspecific adsorption of antibodies on the Fc-receptors on the surface of SC.

These experiments thus revealed marked interlinear differences in the magnitude of the immune response to MPAA. The fact that C57BL/6 and CC57BR mice carrying the same H-2^b haplotype respond in the opposite manner, is evidence of an essential role of genes outside the H-2 complex in the control of the immune response to this antigen. This conclusion is in agreement with data in the literature on control of the immune response to bacterial polysaccharides [4, 7].

Effect of Dose of MPAA on Immune Response in C57BL/6 Mice

Parameter	Dose of antigen during immunization, μg				
		0,01	0,05	0,5	2,5
Number of AFC per spleen	45 (16—126)	208 (78—550)	714 (127—3981)	430 (151—1202)	296 (93—944)
Number of AFC per 10^7 cells	6 (2—21)	27 (11—65)	83 (16—436)	47 (19—112)	39 (11—133)
Number of animals in group	3	5	5	6	5

TABLE 2. Absorption of Anti-MPAA Serum by SC from Mice of Different Lines

Cells used for absorption	Titer of serum	
	before absorption	after absorption
C57BL/6	1:256	1:32
C57BL/6	1:64	1:16
CC57BR	1:256	1:32
CC57BR	1:64	1:16
CBA	1:256	1:32
CBA	1:64	1:16

The intermediate value of the immune response found in the F_1 hybrids compared with responses of the parental lines is evidence against the hypothesis of simple dominance of the characteristic of strength of the immune response to the antigen used. Inheritance of the ability to give an immune response to MPAA is evidently codominant in character, as has also been shown for several other antigens [6].

When discussing the possible causes of the low immune response of C57BL/6 mice to MPAA it must be noted that these data are evidence against a role of antigenic mimicry in determination of the level of the immune response in this test system. Considering data in the literature [4] obtained by a study of control of the immune response to pneumococcal polysaccharide, it can be postulated that the genetic defect in C57BL/6 mice is expressed as reduced activity of T amplifiers or increased generation of specific T suppressors. Another possible cause of the low level of the immune response in C57BL/6 mice may be the small number of clones of B cells carrying specific Ig receptors, and also the insufficient ability of B cells to differentiate into antibody-producing cells under the influence of antigen and (or) regulatory factors. In further experiments the writers will aim to determine the cellular level of expression of genes of the immune response in mice responding in an opposite manner to MPAA.

LITERATURE CITED

1. V. I. Kuvakina et al., Zh. Mikrobiol., No. 4, 63 (1974).
2. A. I. Mokrenko, in: Modern Methods of Experimental Immunology and Different Aspects of Their Application [in Russian], Moscow (1976), p. 131.
3. A. F. Frolov and V. A. Borisov, Immunologiya, No. 3, 63 (1982).
4. P. J. Baker et al., J. Immunogenet., 3, 275 (1976).
5. N. J. Deacon et al., Br. J. Exp. Pathol., 59, 644 (1978).
6. A. Ebringer and N. J. Deacon, J. Immunogenet., 3, 401 (1976).
7. O. Makell et al., Scand. J. Immunol., 12, 155 (1980).
8. P. H. Wooley and A. Ebringer, J. Med. Microbiol., 13, 11 (1980).

SPONTANEOUS AND CONCANAVALIN A-INDUCED PRODUCTION OF LEUKOCYTE MIGRATION INHIBITION FACTOR BY HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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Much attention is now being paid to the control of production of lymphokines and, in particular, of factors influencing migration. Such factors can be produced under the influence of various stimuli, and also spontaneously, under normal conditions [1, 2] and in various diseases [6-8, 11]. It has been shown that cultures of T cells contain an inhibitor of the production of microphage migration inhibition factor (MMIF) [9]. It can be tentatively suggested that a regulatory role in MMIF production is played by suppressor cells [5]. However, the factors and conditions responsible for regulation of production of leukocyte migration inhibition factor (LMIF) have received little study.

The object of this investigation was to study LMIF production by human peripheral blood lymphocytes (PBL) *in vitro*, spontaneously and under the influence of the mitogen concanavalin A (con A).

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