3. E. V. Sidorova, Vopr. Med. Khim., No. 6, 89 (1965).
4. E. V. Sidorova, Usp. Sovrem. Biol., 84, No. 6, 410 (1977).
5. S. Avrameas, J. Antoine, T. Ternynck, et al., Ann. Immunol., 127, 551 (1976).
6. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
7. G. A. Molinaro, E. Maron, W. C. Eby, et al., Eur. J. Immunol., 5, 771 (1975).
8. M. Potter, E. Appella, and S. Geisser, J. Mol. Biol., 14, 361 (1965).
9. I. Rappaport, Cell. Immunol., 6, 473 (1973).
10. P. Valette-Robin, N. Dupont-Mairesse, and R. Jeener, Biochem. Biophys. Res. Commun., 20, 600 (1965).

# IMMUNOLOGIC TOLERANCE TO SURFACE POLYSACCHARIDES OF MENINGOCOCCI OF SEROGROUPS A AND C

A. I. Mokrenko

UDC 576.851.232.097.2:576.851.232.098

KEY WORDS: polysaccharides of meningococcus A and C; immunologic tolerance; antibody-forming cells.

Induction of a state of specific immunologic areactivity to bacterial antigens of polysaccharide nature is a difficult experimental task. For instance, in order to induce immunologic tolerance to the polysaccharide Vi-antigen of *Salmonella typhi* the method used is to treat the animals with antilymphocytic sera [4] or cyclophosphamide [7] in conjunction with injection of relatively high doses of antigen — 200 μg or more. To induce tolerance to levan [9] or the polysaccharide of type III pneumococci [8], doses 250-1000 times larger than the optimal immunizing dose are required.

It was shown previously during a study of the dependence of the immune response of mice on the dose of meningococcal polysaccharides, that the response of animals receiving from 50 to 1000 μg of these antigens was considerably lower than that of mice receiving antigens in doses of 0.005 to 0.5 μg [5].

The object of this investigation was to study whether inhibition of the immune response observed is in fact immunologic tolerance and, if so, to study some particular features of this phenomenon.

## EXPERIMENTAL METHOD

Experiments were carried out on CBA, AKR, and (CBA × C57BL/6)$F_1$ mice and on noninbred albino mice of both sexes weighing 18-20 g. Surface polysaccharides of meningococci of serogroup A (PA), which is a polymer of N-acetyl-O-acetyl-mannosamine phosphate, and polysaccharide from meningococci of serogroup C (PC), which is a polymer of N-acetyl-O-acetyl-neuraminic acid, were used as antigens. Both antigens, which were obtained from the Moscow Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR, by a modified method of Gotschlich et al. [3], had molecular weights of about 100,000.

To induce an immune response, antigens were injected intravenously into the mice and, on the 4th day, the number of antibody-forming cells (AFC) was counted in the spleens by a modification of the standard passive local hemolysis-in-gel technique [6]. The main features of the method were described previously [5]. At the same time as AFC were detected, the antibody titers in the animals' sera were determined by the passive hemagglutination test (PHT).

In some experiments, specificity of tolerance was verified by the use of the polysaccharide Vi-antigen of *S. typhi*. To detect AFC against Vi-antigen, the passive local hemol-

Laboratory of Immunology, Moscow Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 9, pp. 339-341, September, 1980. Original article submitted September 11, 1979.

  

TABLE 1. Specificity of Immunologic Tolerance to Group A and C Meningococcal Polysaccharides in Noninbred Mice

| Material injected into mice | | Number of AFC per spleen (geometric mean and confidence interval)* |
|---|---|---|
| first injection | second injection | |
| Physiological saline | 0.5 μg PA | 1 040 (660—1 640) |
| 25 μg PA | 0.5 μg PA | 182 (115—286) |
| 25 μg PA | Physiological saline | 150 (99—225) |
| 25 μg PA | 0.5 μg Vi-antigen | 16 750 (11 250—24 950) |
| Physiological saline | 0.5 μg Vi-antigen | 15 450 (9 190—26 000) |
| Physiological saline | 0.5 μg PC | 880 (780—1 090) |
| 25 μg PC | 0.5 μg PC | 165 (86—317) |
| 25 μg PC | Physiological saline | 117 (87—158) |
| 25 μg PC | 0.5 μg Vi-antigen | 14 720 (10 400—20 840) |

*In the group of 7 mice.

ysis (plaque) method also was used [1], and serum antibodies were detected by the PHT [2].

All quantitative results obtained were subjected to statistical analysis by Lord's method.

## EXPERIMENTAL RESULTS

In the experiments of series I the immune response of mice of different strains to PA and PC was investigated depending on the dose of antigens injected. In all the animals studied the most intensive response was observed to injection of antigens in a dose of 0.5 μg; an increase in the dose of both PA and PC to 5 μg and, in particular, to 50 μg, led to sharp inhibition of the immune response. This inhibition was reflected both in the number of AFC in the spleens of the mice and in the serum antibody titers.

The results suggested that a single intravenous injection of 5-50 μg of meningococcal polysaccharides induces a state of immunologic tolerance in mice. To test this hypothesis experiments were carried out in which the specificity of inhibition of immunologic reactivity induced by injection of meningococcal polysaccharides was estimated (Table 1).

It will be clear from Table 1 that in mice receiving 25 μg PA or PC 7 days before the test injection (0.5 μg of antigens) the response to homologous antigen was sharply inhibited, but ability to respond to heterologous Vi-antigen of *S. typhi* was unchanged. Consequently, inhibition of the response to meningococcal polysaccharides was specific in character and was an indication of the formation of immunologic tolerance. The phenomenon discovered was independent of fixation of the test dose of antigen by antibodies: In mice receiving only 25 μg PA or PC the number of AFC in the spleen was very small, and no antibodies were present in the serum.

In the next series of experiments the duration of tolerance induced by a single injection of meningococcal polysaccharides was studied. PA was injected intravenously in a dose of 50 μg in CBA mice and a test dose of antigen (0.5 μg) was injected at various times thereafter. As Fig. 1 shows, 10 days after induction of tolerance the number of AFC in the spleens of the tolerant mice receiving 0.5 μg PA was 6.1% of the number in the control group; after 30 days it was 10.7%, and after 45 days 24.2% (the mean absolute numbers for the group were 275, 487, and 1096 AFC per spleen, respectively). In the period between the 45th and 72nd days loss of tolerance was observed and the mice completely regained their ability to respond normally to the particular antigen; after immunization on the 73rd day the response of the tolerant animals to the optimal dose of antigen was 102% of the control. In tolerant mice not receiving the test injection of antigen (but injected with physiological saline), antibody production was absent at all times. A similar dynamics of tolerance also was observed in noninbred mice after injection of 50 μg PA. Tolerance began to be recorded on the 3rd day, but loss of tolerance took place sooner — by the end of the first month.

After loss of tolerance, some strengthening of immunoreactivity was observed. For instance, in (CBA × C57BL/6)$F_1$ mice which were tolerant when tested on the 22nd day, the response to injection of the optimal dose of PA 100 days after induction of tolerance was significantly greater than that in the control group: The mean number of AFC was 3025 (2340-3920), and the serum antibody titer was 1:176 (1:79-1:396), whereas in the control mice the number of AFC was 1446 (933-2240), and the serum antibody titer was 1:88 (1:52-1:152). This

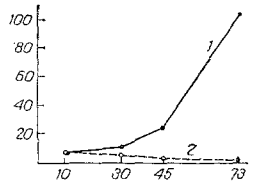


Fig. 1. Duration of tolerance to polysaccharide A in CBA mice. Abscissa, days after induction of tolerance; ordinate; number of AFC in spleen of tolerant mice on 4th day after injection of 0.5 μg antigen (1) or of physiological saline (2) (in % of number of AFC in spleen of control mice receiving the same dose of PA).

fact suggests that this form of tolerance, at least to PA, does not prevent the formation of an immunologic memory, which later, after loss of tolerance, is realized as a response of secondary type to the repeated antigenic stimulus.

These results are evidence that a single intravenous injection of 25-50 μg of meningococcal polysaccharides without immunodepressants induces a state of immunologic tolerance in mice. This form of tolerance is not low-dose tolerance, for the optimal immunizing dose of meningococcal polysaccharides is close to 0.5 μg, but an increase in the dose to 1000 μg leaves the tolerogenic effect unchanged. The nature of this form of immunologic tolerance and its mechanisms require further experimental study.

LITERATURE CITED

1. A. A. Korukova, Byull. Éksp. Biol. Med., No. 12, 103 (1971).
2. N. A. Kraskina and N. M. Gutorova, Tr. Mos. Nauch.-Issled. Inst. Épidemiol. Mikrobiol., 9, 130 (1962).
3. V. I. Kuvakina, M. A. Smirnova-Mutusheva, N. V. Kholchev, et al., Zh. Mikrobiol., No. 4, 63 (1974).
4. T. K. Lopatina, in: Modern Methods of Experimental Immunology and Different Aspects of Their Use [in Russian], Moscow (1976), pp. 17-20.
5. A. I. Mokrenko, in: Modern Methods of Experimental Immunology and Different Aspects of Their Use [in Russian], Moscow (1976), pp. 131-135.
6. L. A. Pevnitskii, L. N. Fontalin, V. V. Solov'ev, et al., in: Proceedings of a Conference on General Immunology [in Russian], Moscow (1965), p. 128.
7. L. N. Fontalin and T. B. Prigozhina, Byull. Éksp. Biol. Med., No. 1, 33 (1979).
8. J. G. Howard, G. H. Cristie, B. M. Courtenay, et al., Eur. J. Immunol., 2, 269 (1972).
9. J. J. Miranda, H. Zola, and J. G. Howard, Immunology, 23, 843 (1972).