DISSOCIATED IMMUNOLOGICAL AREACTIVITY OF MICE TO Vi-ANTIGEN OF Salmonella typhi

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Treatment of mice with a high dose of Vi-antigen and cyclophosphamide altered the immunoreactivity of the animals: in response to injection of a test dose of Vi-antigen marked inhibition of the formation of antibody-forming cells synthesizing both 19S- and 7S-antibodies was observed. The areactivity thus found is specific in character. Meanwhile, a high concentration of antibodies against Vi-antigen can be found by several tests in the serum of these animals.

Combined administration of a high dose of antigen and the powerful immunodepressant cyclophosphamide induces a state of immunological tolerance in adult animals to various corpuscular antigens [2, 4-6, 8]. After combined injection of soluble Vi-antigen of Salmonella typhi and cyclophosphamide, judging from the results of titration of antibodies circulating in the blood stream and detected by the passive hemagglutination test (PHT) with a Vi diagnostic serum, a state phenomenologically similar to tolerance is produced [3].

The object of this investigation was to make a more detailed study of immunological areactivity to Vi-antigen, reproduced with the aid of cyclophosphamide, by the use of several tests.

EXPERIMENTAL METHOD

Adult CBA and (CBA × C57BL)F_1 mice and noninbred albino mice were injected intraperitoneally with 200 or 500 μg Vi-antigen of S. typhi.*. These animals received an intraperitoneal injection of 200 mg/kg cyclophosphamide 42-44 h later. Control animals received either Vi-antigen or cyclophophamide or neither. After 2-3 weeks all the animals were given an intravenous injection of 1 μg Vi-antigen (test injection). The number of antibody-forming cells (AFC) in the spleen and, sometimes also, in the cervical lymph glands was determined 3-4 days later by the passive hemolysis in gel test with sheep's erythrocytes loaded with Vi-antigen [1]. In some experiments the "indirect" plaques were counted by incubating the dishes additionally before introduction of the complement with rabbit antiserum against mouse γ-globulin in a dilution of 1:300 for 1 h. If sheep's erythrocytes were used for the test injection, the AFC were determined by the usual method of local hemolysis in gel as described by Jerne [7].

Antibodies in the sera of the experimental and control animals were determined in the PHT with human group 0 erythrocytes loaded with Vi-antigen (Vi-erythrocytes) or with standard erythrocytic Vi diagnostic serum (Moscow Research Institute of Epidemiology and Microbiology). In some cases the PHT was carried out in a parallel series with sera treated with cysteine.

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TABLE 1. Induction of Immunological Areactivity by S. typhi Vi-antigen in Adult Mice by Combined Injections of Antigen and Cyclophosphamide

Group	Preliminary injections*	Number of mice	Number of AFC in spleen ($\times 10^3$)†	Titers of circulating antibodies in PHT with Vi-erythrocytes
1	200-500 μg Vi-antigen and cyclophosphamide	9	0,2	<2
2	200-500 μg Vi-antigen and cyclophosphamide	42	0,4 (0,3—0,6)	3,9±0,5
3	500 μg Vi-antigen	32	53,4 (40,1—71,9)	8,5±0,4
4	Cyclophosphamide	41	59,3 (42,3—83,2)	7,7±0,2
5	—	43	153,5 (123,3—191,0)	9,2±0,3

*All mice except animals of group 1 received intravenous injection of 1 μg Vi-antigen 2 weeks after preliminary injections (test injection).

† Number of AFC in spleen and titer of circulating antibodies were determined on the 4th day after test injection.

TABLE 2. Titers of Antibodies in PHT with Vi-erythrocytes and with Vi Diagnostic Serum and Their Sensitivity to Cysteine (5th day after test stimulus)

Treatment of mice before test injection	Vi-erythrocytes		Vi-diagnostic serum	
	without cysteine	with cysteine	without cysteine	with cysteine
200-500 μg Vi-antigen + cyclophosphamide	7,9±0,7 (9)	1,8±0,8 (9)	2,6±0,9 (9)	1,8±0,5 (9)
Cyclophosphamide	11,4±0,3 (8)	5,0±0,8 (8)	7,9±0,7 (8)	4,5±0,6 (8)
200-500 μg Vi-antigen	10,4±0,6 (7)	8,1±0,5 (7)	8,0±0,8 (7)	6,1±0,8 (7)
—	11,4±0,3 (8)	5,9±0,4 (8)	8,0±0,3 (8)	4,1±0,3 (8)

Note. Titers of antibodies expressed in logarithms to base 2; initial dilution 1:10 (1.0). Number of samples in parentheses. Each sample a mixture of sera from 5 mice.

The numerical results were subjected to statistical analysis: the geometric mean and its confidence limits ($P < 0.05$) were calculated and the significance of the differences between the mean values of the compared group was determined by Student's method.

EXPERIMENTAL METHOD

Induction of tolerance in animals with a large dose of Vi-antigen and cyclophosphamide, if carried out 2 weeks before the test injection, sharply reduced the immunoreactivity of the animals as shown by the number of AFC in the spleen on the 4th day after test injection (Table 1, group 2). Administration of either a large dose of antigen or cyclophosphamide separately to the mice led to only a very slight inhibition of immunoreactivity (groups 3 and 4). The phenomenon was characterized by immunological specificity, as shown by comparing the responses of mice in which tolerance had been induced by testing with Vi-antigen and with sheep's erythrocytes (Fig. 1).

The results of titration of antibodies circulating in the blood stream in the PHT with Vi-erythrocytes for the experimental animals on the 5th day after the test injection of Vi-antigen were unexpected (Table 2). These mice were found to have antibodies in relatively high concentration, obviously not corresponding to the small numbers of AFC in the spleen. These antibodies were mainly cysteine-sensitive, i.e., they belonged to the 19S-class. Parallel titration of the same sera in the PHT with the Vi diagnostic serum con-

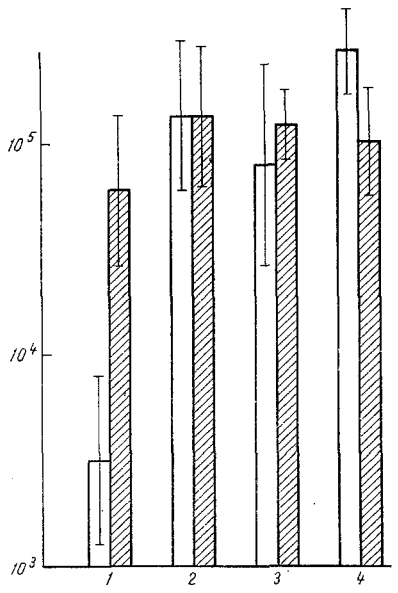

Fig. 1

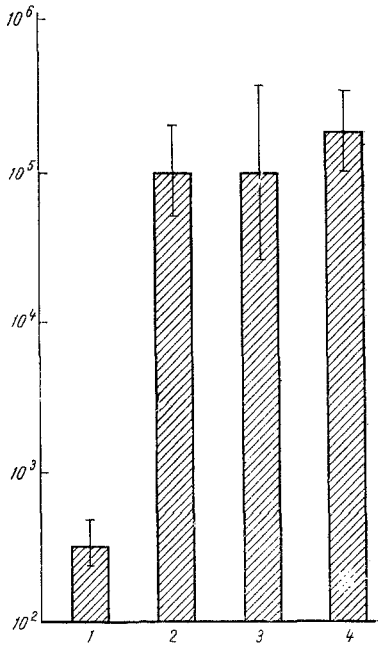

Fig. 2

Fig. 1. Specificity of areactivity induced by combined injection of a high dose of Vi-antigen and cyclophosphamide: 1) mice receiving preliminary injection of Vi-antigen and cyclophosphamide; 2) mice receiving cyclophosphamide alone; 3) mice receiving preliminary injection of Vi-antigen (high dose) alone; 4) intact mice. Unshaded columns represent number of AFC in spleen on the 4th day after test injection of Vi-antigen; shaded columns show number of AFC in spleen on the 4th day after test injection of sheep's erythrocytes. Ordinate: number of AFC.

Fig. 2. Number of AFC synthesizing 7S-antibodies in spleen of mice on the 4th day after test injection of Vi-antigen: 1) mice receiving preliminary injections of Vi-antigen and cyclophosphamide; 2) mice receiving preliminary injection of cyclophosphamide; 3) mice receiving preliminary injection of Vi-antigen (high dose); 4) control mice. Ordinate, number of AFC.

firmed earlier results [3]: all sera had lower titers than 1:40 in this test. The reasons for disagreement between the results of titration with Vi-erythrocytes and Vi-diagnostic serum, prepared with the aid of formalinized erythrocytes, require further study.

The individual values of the titers of antibodies detectable in the PHT with Vi-erythrocytes in the experimental animals varied within very wide limits: on the 4th day after the test injection of 1 μg Vi-antigen intravenously, the titers of 15 of the 42 mice tested were below 1:20, in 9 animals they were between 1:20 and 1:160, and in 18 between 1:320 and 1:2560. It must be emphasized, in particular, that before the test injection (Table 1, group 1) antibodies were not found serologically in the blood of these mice either with Vi-erythrocytes or with the Vi-diagnostic serum.

The possible reasons for the discrepancy between the numbers of AFC in the spleen and the titer of antibodies circulating in the blood stream may be as follows:

1) extrasplenic synthesis of antibodies;

2) an earlier than usual maximum of the number of AFC;

3) different properties of the antibodies detected by the passive local hemolysis in gel test and the PHT;

4) production of a subthreshold quantity of antibodies by 1 AFC, not detectable by the test; in total, however, a sufficiently high concentration of antibodies is formed.

Determination of the total number of AFC in the cervical lymph glands on the 4th day after the intravenous test injection of Vi-antigen revealed a small number of AFC in the animals of all the experimental groups, the smallest number being found in the experimental mice (mean 33 AFC). Mice previously receiving cyclophosphamide alone had 81 AFC in their lymph glands, and those receiving nothing before the test injection had 104 AFC.

Investigation of the number of AFC in the spleen on the 3rd day after the intravenous test injection showed that the mean number of AFC in the mice in which tolerance had been induced was 469. Meanwhile, in the control mice (previously receiving cyclophosphamide alone or receiving nothing) the mean number of AFC in the spleen was 1009 and 2985 respectively.

These results suggest that the relatively high blood level of antibodies in the animals in which tolerance had been induced was not due to the earlier maximum of the immune response or to extrasplenic antibody synthesis.

Determination of the "indirect" plaques (7S-AFC) showed that the number of AFC producing 7S-antibodies was reduced in the spleen of the experimental mice after the test injection of Vi-antigen (Fig. 2), in good agreement with earlier observations (Table 2) on the cysteine-sensitivity of the antibodies in the experimental animals.

The results thus demonstrate that the induction of tolerance in mice by a high dose of Vi-antigen and cyclophosphamide changes the immunoreactivity of the animals: after the intravenous test injection of Vi-antigen, the formation of AFC in the spleen is sharply inhibited. Meanwhile, the serum of these animals contains a high concentration of antibodies, not matching the number of AFC. In order to understand the discrepancies observed between the assessments of the response of the experimental animals to the test stimulus, further experimental investigations are necessary.

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