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SPECIFIC BLOCKAGE OF THE IMMUNE RESPONSE BY AN EXCESS OF Salmonella typhi Vi-ANTIGEN

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Intravenous injection of 200 μg of Vi-antigen into adult mice induces a state of short-term (10-12 days) areactivity in the animals. The observed depression of the immune response is due to blockage of the immunocompetent cells and not to masking of antibody production through binding with the excess of free antigen. Washing a suspension of spleen cells twice before using them in the local passive hemolysis in gel test did not reduce the blockage of the immune response; moreover, no free antigen capable of binding antibodies produced by cells of the immune animal could be found in the spleen of the experimental animals. Blockage of the immune response could be abolished by injecting heterologous antiserum against Vi-antigen into the animals 18-24 h before Jerne's test. Injection of 6-thioguanine after 200 μg of Vi-antigen prevented restoration of the immune response by means of the antiserum. It is concluded from the results that injection of a massive dose of Vi-antigen does not block proliferation and differentiation of antigen-recognizing cells but inhibits the synthesis or secretion of antibodies.

KEY WORDS: Vi-antigen; tolerance; immunologic paralysis.

Injection of a large dose of polysaccharide antigens induces immunologic paralysis in mice [6-8]. The mechanism of this phenomenon has been adequately studied for paralysis induced by the polysaccharide of type III pneumococcus and by other antigens of similar structure [7, 8]. The form of areactivity observed has been shown to be due to blockade of lymphocytes by antigen and not to binding of the antibodies by the excess of antigen (peripheral neutralization). With respect to the Vi-antigen of Salmonella typhi it is known that injection of a massive dose of this polysaccharide into mice sharply reduces the blood antibody titers [1, 3].

The object of this investigation was to study whether this effect is due to peripheral neutralization of antibodies or to blockade of lymphocytes by the antigen.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male mice weighing 18-20 g. A commercial preparation of S. typhi Vi-antigen produced by the Moscow Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR, was used as the antigen [4].

The experimental mice were given an intravenous injection of 200 μg Vi-antigen and the controls an injection of 10 μg of the same antigen (the optimal immunogenic dose). The number of antibody-forming cells (AFC) in the spleen was determined 4 days later by the passive hemolysis in gel method (Jerne's test) using sheep's red cells loaded with Vi-antigen [2]. Hyperimmune rabbit sera against Vi-antigen were obtained by injecting 100-200 μg Vi-antigen at monthly intervals several times into rabbits. Blood was taken on the seventh

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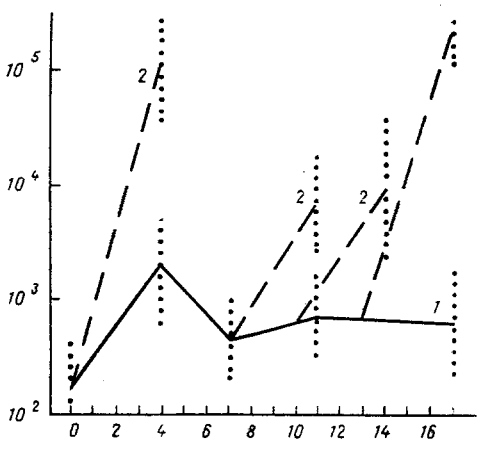

Fig. 1

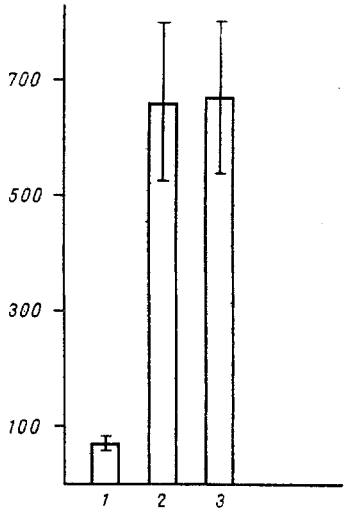

Fig. 2

Fig. 1. Dynamics of immune response to 200 μg Vi-antigen and dynamics of restoration of ability to give immune response to test dose (10 μg) of Vi-antigen. 1) Immune response after injection of 200 μg Vi-antigen; 2) immune response to test injection of 10 μg Vi-antigen. Abscissa, days after intravenous injection of 200 μg Vi-antigen; ordinate, number of AFC in spleen.

Fig. 2. Results of combined incubation of spleen cells of mice immunized with 200 and 10 μg Vi-antigen. 1) Spleen cells ($5 \cdot 10^6$) of mice receiving 200 μg Vi-antigen together with cells of intact animals ($50 \cdot 10^6$); 2) spleen cells ($5 \cdot 10^6$) of mice receiving 10 μg Vi-antigen together with cells of intact animals ($5 \cdot 10^6$); 3) $5 \cdot 10^6$ spleen cells of animals receiving 200 μg Vi-antigen together with the same number from animals receiving 10 μg Vi-antigen. Ordinate, number of AFC.

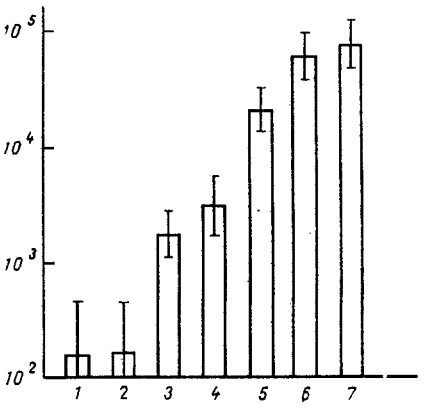

Fig. 3. Restoration of immune response to Vi-antigen with the aid of specific antiserum. 1) Intact mice; 2) mice receiving rabbit antiserum against Vi-antigen; 3) mice receiving 200 μg Vi-antigen; 4) mice receiving 200 μg Vi-antiserum and normal rabbit serum; 5) mice receiving 200 μg Vi-antigens and rabbit antiserum against Vi-antigen; 6) mice receiving 10 μg Vi-antigen; 7) mice receiving 10 μg Vi-antigen and rabbit antiserum against Vi-antigen. Sera injected 18-24 h before animals were killed. Ordinate, number of AFC in spleen on fourth day after injection of antigen.

TABLE 1. Effect of 6-TG on Restoration of Immune Response to Vi-Antigen by Means of Antiserum

Treatment of animals			Number of mice	Number of cells in spleen (millions)	Number of AFC per 10^6 nucleated cells	Number of AFC in spleen
day 0	day 1	day 3				
200 μg Vi-antigen	—	—	13	223 (177—269)	9 (7—12)	1 714 (1 133—2 580)
The same	—	Antiserum	10	259 (211—307)	50 (30—84)	12 166 (7 262—20 368)
6-TG, 200 μg Vi-antigen	—	—	7	86 (66—106)	8 (4—16)	360 (156—936)
The same	—	Antiserum	11	78 (53—103)	49 (25—93)	3 536 (1 644—7 585)
200 μg Vi-antigen	6-TG	Antiserum	12	89 (63—115)	⩽2 (1—4)	⩽190 (183—198)
10 μg Vi-antigen	—	—	12	265 (184—346)	123 (74—205)	27 509 (16 222—46 548)
6-TG, 10 μg Vi-antigen	—	—	10	73 (55—91)	276 (182—418)	18 475 (10 158—30 090)
10 μg Vi-antigen	6-TG	—	14	88 (69—107)	3 (2—4)	228 (140—374)

Legend. Mean values and confidence intervals (in parentheses) shown in table.

day after the last immunization and the animals were used over and over again. The sera were heated for 30 min to 56°C, frozen, and kept at −20°C. The titer of the sera was IgM 1:32,000 and IgG 1:640. Injections of 0.5 ml of antiserum were given intravenously to the mice.

6-Thioguanine (6-TG) was injected intravenously, in a dose of 12 mg/kg body weight, 3 h before or 24 h after injection of the antigen. 6-TG was diluted in a small volume of spirit of ammonia, boiled for a few minutes to remove the excess of ammonia, and then diluted with physiological saline to a concentration of 1.2 mg/ml.

Student's criterion was used for the statistical analysis.

EXPERIMENTAL RESULTS

Intravenous injection of 200 μg of Vi-antigen induced a weak immune response in the mice. The number of AFC in the spleen was 1/20-1/30 of that after injection of the optimal dose of antigen (10 μg). As Fig. 1 shows, the low level of the response to the larger dose of antigen was accompanied by refractoriness to subsequent injection of the optimal immunogenic dose. Depression of ability to give an immune response was of short duration, and 10-13 days later ability to respond to 10 μg was fully restored.

Blockade of the immune response can be explained: a) by peripheral neutralization of antibodies by the excess of antigen persisting in the body; b) by reversible blockade of immunocompetent B lymphocytes by the antigen; c) by elimination of clones of immunocompetent cells on account of exhaustive differentiation.

True peripheral neutralization was not observed after injection of 200 μg Vi-antigen, for not only was the titer of antibodies against Vi-antigen in the blood serum lowered, but the number of AFC in the spleen also was reduced, and this is not characteristic of peripheral neutralization. However, an attempt was made to verify whether the decrease in the number of AFC in the experimental animals was connected with selective accumulation of large quantities of Vi-antigen in the spleen.

Washing the spleen cells twice before carrying out Jerne's test did not restore the immune response of the mice to Vi-antigen. In addition, unwashed spleen cells suspended in a small volume of medium were incubated with cells synthesizing antibodies against Vi-antigen intensively. No inhibition of antibody synthesis (a decrease in the number of AFC) was observed (Fig. 2). Consequently, blockade of the immune response was not due to the inhibitory action of free antigen able to migrate into the agar during Jerne's test simultaneously with spleen cells of the experimental animals and to compare with Vi-erythrocytes for antibodies.

It can be concluded from the results of this experiment that even if an excess of Vi-antigen is present in the spleen, this antigen is mainly bound with lymphocytes.

The results of both experiments showed that the quantity of free antigen in the spleen of animals receiving a massive dose of Vi-antigen was insufficient to mask antibody production.

The blockade of the immune response could be abolished with the aid of hyperimmune rabbit serum against Vi-antigen if this was injected 18-24 h before the number of AFC in the spleen was determined. As

Fig. 3 shows, injection of 0.5 ml antiserum on the third day after 200 μg Vi-antigen and 24 h before the local hemolysis in gel test restored the immune response quickly. The number of AFC in the spleen was increased tenfold, i.e., it was close to the response to 10 μg antigen. The increase in the number of AFC could not be attributed to the appearance of false plaques on account of liberation of the injected antibodies from macrophages ingesting them by pinocytosis, for the background level of the immune response to Vi-antigen did not increase after injection of antiserum into intact animals.

Abolition of blockade of the immune response was specific in character: It could not be obtained with the aid of intact rabbit serum, and injection of antiserum against Vi-antigen did not stimulate the immune response either to 10 μg Vi-antigen or to sheep's red cells.

The results of these experiments show that blockade of the immune response induced by 200 μg Vi-antigen is reversible and, consequently, it is unconnected with elimination of the immunocompetent clones. However, it remains to be explained whether the form of reactivity observed is connected with blockade of the proliferation of antigen-recognizing cells or with a disturbance of the synthesis of secretion of antibodies. An attempt was made to solve this problem by eliminating proliferating cells by means of the immunodepressant 6-TG before injection of the antiserum (Table 1).

6-TG caused a sharp decrease in the total number of cells in the spleen as a result of death of cells dividing at the moment of its injection.

If 6-TG was given before the antigen, however, its action was significantly weaker.

As Table 1 shows, in mice receiving 6-TG before the massive dose of antigen, antiserum against Vi-antigen restored the immune response, but in animals receiving 6-TG after 200 μg of Vi-antigen, restoration of the immune response with the aid of antiserum was not observed.

It can be concluded from the experiments that precursors of "latent" AFCs (detectable with the aid of specific antiserum) proliferate intensively after contact with the antigen.

Several workers have recently shown that incubation of a hapten–polysaccharide complex with AFC synthesizing antibodies against the hapten leads to specific inhibition of antibody secretion [9, 10]. Blockade of AFC is induced in this case by fixation of antigen by the immunoglobulin receptors of the lymphocytes [5]. Blockade of the AFC by Vi-antigens probably takes place by a similar mechanism. It is also impossible at present to rule out another explanation: the possibility of intracellular binding of synthesized antibodies with antigen deposited in the cell. The secretion of antibodies in the form of an antigen–antibody complex may hinder detection of AFC.

These problems require further study. However, it is very likely that the main cause of areactivity to Vi-antigen induced by injection of a massive dose of Vi-polysaccharide is blockade of AFC by the antigen.

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