CREATION OF IMMUNOLOGICAL TOLERANCE TO SOLUBLE POLYSACCHARIDE ANTIGEN IN MICE WITH THE AID OF ANTILYMPHOCYTIC GLOBULIN

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Treatment of (CBA × C57BL) F_1 mice receiving a large dose of soluble polysaccharide Vi-antigen of Salmonella typhi with horse antimouse lymphocytic globulin leads to the development of specific immunological tolerance to this antigen. This phenomenon is based on a true change in the reactivity of the immunocompetent cells, for spleen cells of animals tolerant to Vi-antigen, when exposed a second time to contact with the antigen, did not form antibodies in the irradiated recipient, unlike spleen cells of intact animals.

The prospect of creating stable immunological tolerance in the adult is particularly interesting because the solution of this problem would allow considerable progress to be made toward overcoming the difficulties concerned with transplantation of organs and tissues. The experimental and clinical study of the properties of antilymphocytic preparations has demonstrated that they can be used to form immunological tolerance. Tolerance to a skin graft can be induced by injection of lymphoid cells or of cell-free tissue extracts from the donor in conjunction with antilymphocytic serum [8-10]. The creation of immunological tolerance is facilitated by the combined use of antilymphocytic sera and chemical immunodepressants [11], irradiation [8], and thymectomy [6]. There is little information in the literature on the effect of antilymphocytic sera on the formation of tolerance to purified protein or polysaccharide antigens. Lance [7] observed a specific decrease in immunological reactivity in mice receiving bovine serum albumin in conjunction with antilymphocytic serum.

The object of the present investigation was to study the effect of antilymphocytic antibodies on the ability of the bacterial polysaccharide Vi-antigen of Salmonella typhi to induce immunological tolerance in mice.

EXPERIMENTAL METHOD

Antilymphocytic globulin (ALG) was obtained by salt fractionation from the serum of a horse immunized repeatedly with the lymphocytes, spleen, and thymus of mice. The antilymphocytic serum was first inactivated by heating to 56°C for 30 min and absorbed by repeated incubation with washed mouse erythrocytes. The titer of ALG in the hemagglutination test did not exceed 1:4, in the lymphagglutination test it varied from 1:320 to 1:640, and in the cytotoxic test it varied from 1:1000 to 1:2000. The globulin was injected intraperitoneally into mice, the dose being calculated on the basis of protein content.

Immunization of (CBA × C57BL)F_1 mice with a soluble polysaccharide bacterial antigen, namely the Vi-antigen of S. typhi, was used as the experimental model [1, 5]. The Vi-antigen was injected intravenously, and the sera of the mice were tested for the presence of antibodies against Vi-antigen in the passive hemagglutination test [2].

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TABLE 1. Titer of Antibodies in Mice after Reacting Immunization with Vi-Antigen of S. typhi

Group No.	Treatment of mice: ALG	Treatment of mice: Vi-antigen (in μg)*	Number of mice	Percentage of animals with different titers of antibodies: <1:10	1:10—1:20	1:40—1:80	1:160—1:320	1:640—1:1280	1:2560—1:5120	>1:5120	Mean titer ($\bar{x}_g$) of Vi-antibodies with 95% confidence limits
1	+	20—50	30	0	7	20	40	23	7	3	1:281 (1:118—1:671)
2	+	200	43	47	9	25	19	0	0	0	1: 24 (1: 14—1: 39)
3	+	800—2000	22	9	41	36	9	5	0	0	1: 33 (1: 18—1: 61)
4	—	20—50	19	0	0	5	32	32	21	10	1:760 (1:336—1:1718)
5	—	200	16	0	0	31	38	31	0	0	1:295 (1:195—1:447)
6	—	800—2000	21	5	0	33	38	19	5	0	1:150 (1: 69—1:327)
7	—	—	40	0	2	8	35	35	20	0	1:423 (1:219—1:843)

*In response to primary immunization.

TABLE 2. Ability of Mouse Spleen Cells to Give Immune Response to Vi-Antigen of S. typhi in Irradiated Recipients

No. of group of recipients	Donors of spleen cells	Number of recipient mice	Number of recipient mice with different antibody titers: <1:20	1:40—1:80	1:160—1:320	1:640—1:1280	1:2560—1:5120	Mean titer ($\bar{x}_g$) with 95% confidence limits
1	Intact	17	0	0	2	12	3	1:1040 (1:770—1:1396)
2	Tolerant	25	16	9	0	0	0	1: 11 (1: 8—1: 16)
3	—	20	20	0	0	0	0	0 —

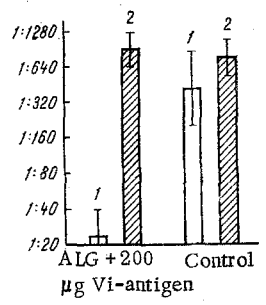

Fig. 1. Specificity of immunological tolerance to Vi-antigen of S. typhi in mice: 1) antibodies against Vi-antigen; 2) antibodies against sheep's erythrocytes. Ordinate, antibody titer.

The main experiments were carried out as follows. The mice received four injections of ALG in a dose of 5-10 mg protein per injection, 2 days and 5 h before injection of the Vi-antigen and 1 and 3 days thereafter. The control animals received no treatment other than immunization. Vi-antigen was injected in doses ranging from 20 to 2000 μg. One month after the first injection of Vi-antigen all the groups of animals received a test injection of an optimal dose of Vi-antigen (0.1 μg). On the 6th day after the reacting immunization, the sera of the mice were tested for the presence of antibodies.

The method of transplantation of lymphocytes described fully in earlier papers by Kraskina and Levenson [3, 4] was used on this occasion. Spleens of donor mice were compressed in a glass homogenizer with the addition of a small volume of medium no. 199. The resulting cell suspension was filtered through two layers of Kapron and injected, into recipients previously irradiated in a dose of 700 R, intraperitoneally in a dose of 150,000,000 cells per mouse. The next day the recipients mice received a test injection of Vi-antigen (0.1 μg). Four days later the sera of the mice were tested for the presence of antibodies.

EXPERIMENTAL RESULTS

The results (Table 1) showed that combined treatment with ALG and a large dose of Vi-antigen sharply reduced the ability of the mice to respond by antibody formation to a second injection of Vi-antigen. Injection of Vi-antigen alone in doses of 20-50 and 200 μg (groups 4 and 5) did not lead to inhibition of the immunological response, and only larger doses of the antigen (group 6) caused some decrease in the antibody titer compared with the control. Conversely, in conjunction with the action of antilymphocytic antibodies, 200 μg of Vi-antigen was enough to cause a sharp decrease in antibody production in response to the test injection (groups 2 and 3).

The resulting immunological tolerance was specific in character, for mice tolerant to Vi-antigen responded by equally intensive antibody production to the injection of 3×10^8 sheep's erythrocytes as did control animals (Fig. 1).

The immunological tolerance of the mice was not the result of the continued immunodepressant action of ALG, for mice treated with globulin alone and receiving the test dose of Vi-antigen 1 month later gave a response indistinguishable from that of the control.

The phenomenon of immunological tolerance must be based on a true change in the reactivity of the immunocompetent cells. To verify whether the phenomenon actually obtained corresponded to this condition, experiments were carried out in which spleen cells of tolerant and intact animals were transplanted into irradiated recipients, which subsequently received an injection of Vi-antigen. The results of these experiments showed (Table 2) that tolerance to Vi-antigen obtained by means of ALG is true tolerance. In the overwhelming majority of mice receiving spleen cells of tolerant animals, Vi-antigen evoked no antibody production whatsoever (group 2), while in the other mice antibodies were present in low titers (1:40-1:80). Conversely, in the group of mice receiving intact spleen cells and Vi-antigen (group 1) there were virtually no animals with low antibody titers. The mean antibody titer in the recipients of the tolerant spleen was 1:11, and in the recipients of the intact spleen it was 1:1040.

These results show that the immunological areactivity to the Vi-antigen of S. typhi obtained with the aid of ALG satisfies the criteria of immunological tolerance: a) it is specific in character, and b) the phenomenon is based on a true change in the reactivity of the immunocompetent cells.

The ALG used in this investigation was obtained by immunizing a horse with a mixture of lymphocytes taken from different lymphoid organs of mice, mainly the thymus and spleen. As the next step it will be interesting to compare the formation of immunological tolerance to Vi-antigen of S. typhi under the influence of antilymphocytic sera of different origin (thymus, spleen, lymph glands, bone marrow). Such an investigation could provide new information on the nature of the phenomenon of immunological tolerance.

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