IMMUNE RESPONSE TO *Salmonella typhi* Vi-ANTIGEN AND TOLERANCE TO IT IN T CELL DEPRIVED MICE

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The role of T suppressors in the regulation of the immune response to *Salmonella typhi* Vi-antigen was studied by comparing the immune response to this antigen in T cell deprived mice (B mice) with the immune response in intact animals. Deprivation of T cells was produced by thymectomy or by lethal irradiation and subsequent injection of embryonic liver cells into the mice. The level of the immune response to Vi-antigen was almost identical in the B mice and control animals. The absence of enhancement of the immune response in the B mice shows that not only induction but also regulation of the immune response to the optimal dose of Vi-antigen is thymus-independent in character. Tolerance to Vi-antigen could be induced in B mice by means of cyclophosphamide; the degree of specific inhibition of the immune response, moreover, was the same as in animals possessing T cells. This suggests that the cause of drug-induced tolerance to this antigen is not activation of T suppressors, but rather a true deficiency of immunocompetent clones of B cells.

KEY WORDS: Vi-antigen; immune response; immunologic tolerance; cyclophosphamide; T suppressors.

Induction of the immune response to certain antigens takes place without the participation of T cells. Such antigens are called thymus-independent. However, the role of T cells in the regulation of the immune response to these antigens remains unclear. According to the observations of Lake and Reed [10], T suppressors limit the immune response to polyvinyl pyrrolidone. T suppressors have also been found in the presence of low-zone tolerance to the polysaccharide of type III pneumococcus [6] and in tolerance to *Salmonella typhi* Vi-antigen induced with the aid of antilymphocytic serum [2]. Meanwhile, many workers have been unable to show that T cells participate in the regulation of the immune response to pneumococcal polysaccharide or to other thymus-independent antigens, and also in most forms of areactivity to polysaccharide antigens: immunologic paralysis, reversible blocking of the immune response, areactivity due to exhausting differentiation of immunocompetent cells [8, 15].

The use of cyclophosphamide (CP) in conjunction with a massive dose of antigen enables immunologic tolerance to be obtained to both thymus-dependent and thymus-independent antigens. It is usually considered that areactivity induced by CP is due to a deficiency of immunocompetent clones [5, 7]. Recently, however, some workers have associated it with activation of T suppressors [12].

The object of the present investigation was to study the role of T cells in the regulation of the immune response to thymus-independent *Salmonella typhi* Vi-antigen and in the formation of tolerance to it, induced by means of CP.

EXPERIMENTAL METHOD

Male (CBA × C57BL/6)F_1 mice weighing 18-20 g were used. The animals were first thymectomized to eliminate T cells. The operation was performed by electric suction under hexobarbital anesthesia (100 mg/kg, intraperitoneally). The mice were irradiated three weeks later in a dose of 1000 R on the EKU-50 apparatus. The source of radiation was ^{60}Co (dose rate 77 R/min). Each irradiated animal received an intravenous injection of 5×10^6-1×10^7

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TABLE 1. Comparison of Intensity of Immune Response of B and TB Mice and of Intact Animals to SRBC, *E. coli* LPS, and *S. typhi* Vi-antigen

Antigen	Animals	Number of animals	Number of AFC in spleen
SRBC (5×10^8 per mouse)	B mice	20	4 272 (1 930—9 470)
	TB mice	20	101 766 (70 962—146 209)
	Intact	18	140 977 (119 470—166 242)
LPS E. coli (10 μg per mouse)	B mice	9	88 965 (37 186—93 675)
	TB mice	10	30 042 (17 580—51 402)
	Intact	10	42 700 (21 677—83 958)
S. typhi vi-antigen (10 μg per mouse)	B mice	14	12 508 (9 628—16 866)
	TB mice	14	24 563 (13 645—44 157)
	Intact	10	23 956 (13 851—44 400)

Legend. Geometric mean values and confidence intervals given. Number of AFC in spleen determined on 4th day after immunization.

liver cells from 17-19-day syngeneic mouse embryos. The embryonic liver at these times is known to contain neither mature T cells nor their precursors capable of maturing in the absence of intact thymus or thymus hormones [13]. The experiments were carried out 1.5-2 months after irradiation of mice (B mice) treated as described above.

At the end of each experiment all the mice were autopsied to verify completeness of removal of the thymus. Animals with remains of the thymus were rejected. Nonthymectomized mice, irradiated and then receiving an injection of embryonic liver cells together with 5×10^7 syngeneic thymocytes (TB mice), and intact animals, served as the controls.

The basic antigen used was *Salmonella typhi* Vi-antigen. To induce an immune response 10 μg Vi-antigen was injected intravenously, and 42-46 h later CP (250 mg/kg per mouse) was injected intraperitoneally. The number of antibody-forming cells (AFC) in the spleen of the mice was counted on the fourth day after injection of the optimal dose of Vi-antigen by the method of local passive hemolysis in gel. In the experiments with adoptive transplantation of lymphocytes the number of AFC in the spleen of the recipients was determined on the fifth day. Details of the methods used were given previously [4].

In some experiments the animals were immunized with sheep's red blood cells (SRBC) in a dose of 5×10^8 SRBC per mouse or with lipopolysaccharide (LPS) of *Escherichia coli* (from Difco) in a dose of 10 μg per mouse. The number of AFC to SRBC and to *E. coli* LPS in the spleen of the experimental animals was determined on the fourth day by the method of local hemolysis in gel [9] and the method of local passive hemolysis in gel [11].

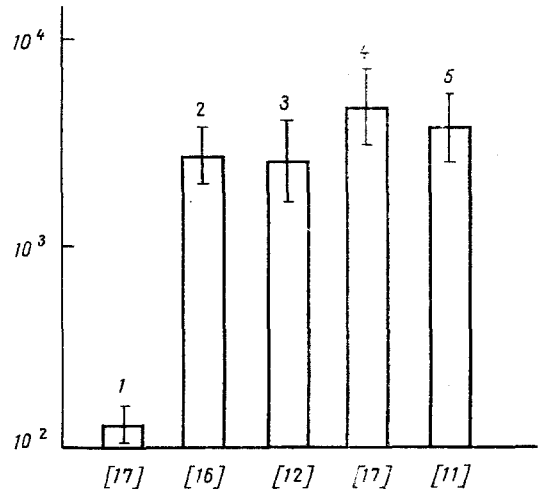

Fig. 1

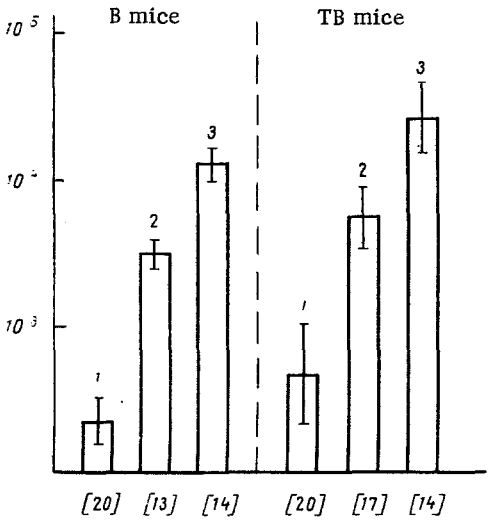

Fig. 2

Fig. 1. Level of immune response to Vi-antigen in irradiated mice each receiving 6×10^7 spleen cells from tolerant donors (1), intact donors (2), and B mice (3), or a mixture of equal numbers (6×10^7) of spleen cells of tolerant donors with intact (4) or B lymphocytes (5). Abscissa, number of animals in group, in parentheses; ordinate, number of AFC in spleen of experimental mice on fifth day after transplantation.

Fig. 2. Level of tolerance to Vi-antigen in B and TB mice two weeks after tolerogenic treatment. Abscissa, number of mice in group, in parentheses; ordinate, number of AFC in spleen on 4th day after injection of test dose of Vi-antigen. 1) Tolerant animals; 2) mice receiving CP; 3) intact mice.

EXPERIMENTAL RESULTS

In the experiments of series I the immunocompetence of the B and TB mice and of intact animals relative to *S. typhi* Vi-antigen and to two control antigens — thymus-dependent (SRBC) and thymus-independent (*E. coli* LPS) — was compared. As Table 1 shows, the level of immune response to SRBC in the B mice was much lower than in the control animals. On immunization with *E. coli* LPS and *S. typhi* Vi-antigen, no significant differences could be found between the number of AFC in the B mice and in the animals of the other two groups. The fact that T cell deprivation in B mice did not disturb the immune response to Vi-antigen is evidence in support of its thymus-independence. Similar results have been obtained by other workers who also studied thymus-dependence of Vi-antigen, but used different methods of eliminating the T cells [1, 14].

Stimulation of the immune response to Vi-antigen in the B mice compared with the response of the TB animals could not be found. On the contrary, a small decrease (by half) in the number of AFC to Vi-antigen was observed in the B mice compared with the number of AFC in the TB animals. The immune response to Vi-antigen of the irradiated syngeneic recipients of spleen cells of B mice did not differ from the response of the animals receiving lymphocytes of intact donors (Fig. 1). Similar results were obtained by Kraskina [1], who used a different method to eliminate T cells: treatment of a suspension of intact lymphocytes with heterologous anti-T serum and complement before adoptive transplantation. These facts are evidence against participation of T suppressors in the regulation of the immune response to Vi-antigen.

In the next part of the investigation the role of T cells in the formation of immunologic tolerance to Vi-antigen induced by CP was studied.

In the experiments of series I cells of tolerant and control animals were injected two weeks after tolerogenic treatment either together or separately into lethally irradiated recipients, which were then immunized with Vi-antigen.

As Fig. 1 shows, the number of AFC to Vi-antigen in mice receiving only cells of tolerant donors after irradiation was only 1/16-1/19 of their number after transplantation of spleen cells from B mice or intact donors. Addition of an equal number of cells of tolerant donors to the B lymphocytes did not depress the recipients' immune response compared with that of mice receiving B lymphocytes alone.

These results are in good agreement with the writers' previous findings, showing absence of "infectiousness" of tolerance to Vi-antigen obtained with the aid of CP [4], and they are evidence against activation of T suppressors in this form of areactivity.

In the next series of experiments a comparative analysis was made of the effectiveness of tolerogenic treatment of B and TB mice. Injection of CP alone depressed the immunocompetence of the B and TB mice relative to Vi-antigen somewhat (Fig. 2). Combined injection of Vi-antigen and CP led to the almost total loss of ability of both B and TB mice to respond to the test dose of Vi-antigen. Injection of Vi-antigen alone (without CP) did not depress immunoreactivity at the times specified [3].

It can be concluded from the results of these experiments that T suppressors do not play an essential role either in the regulation of the immune response to the optimal dose of Vi-antigen or in the formation of tolerance to it, induced by means of CP. The possibility cannot be ruled out that in certain other methods of tolerogenic treatment T suppressors may participate in the specific depression of the immune response to Vi-antigen. However, tolerance to Vi-antigen induced by CP is evidently the result of direct elimination or inactivation of corresponding clones of B cells.

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