INDUCTION OF IMMUNOLOGIC TOLERANCE TO THYMUS-DEPENDENT ANTIGEN (SHEEP'S RED BLOOD CELLS) IN THE ABSENCE OF T CELLS

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The role of T cells in the induction of tolerance of B cells to sheep's red blood cells (SRBC) by means of cyclophosphamide (CP) was investigated. Tolerance was obtained in adult intact mice, in mice irradiated lethally and protected with syngeneic embryonic liver cells and thymocytes (TB mice), and in mice deprived of T cells — either thymectomized or lethally irradiated and protected with embryonic liver cells (B mice). This form of tolerance was shown to be due to specific elimination of T lymphocytes and, to some extent also, of B lymphocytes. Tolerogenic treatment of B mice, as also of TB mice, led to depression of their immunoreactivity. Spleen cells of tolerant B mice did not suppress the immune response of intact spleen cells. It is concluded that under the conditions investigated, tolerance of B cells can be formed without the participation of T lymphocytes.

KEY WORDS: immunologic tolerance, B lymphocytes, T helpers, T suppressors.

The problem of the role of T cells in the formation of tolerance of B cells is not yet clearly understood. According to some data [9, 10] tolerance of B cells both to thymus-dependent and to thymus-independent antigens does not arise in the absence of T cells, indirect evidence of a possible role of T suppressors in this process. However, other workers [8, 12, 13] have found that many thymus-dependent and thymus-independent antigens can induce tolerance in pure populations of B cells. These contradictions can be explained either by the presence of a certain number of T cells, or by the nonspecific effect of the thymus and of T cells on differentiation of B cells, or by differences in the mechanism of different forms of tolerance.

The object of this investigation was to compare the formation of tolerance to a thymus-dependent antigen (sheep's red blood cells — SRBC) in a population of B cells in the presence and absence of T cells. With this approach it was possible to determine not only the need for the presence of T cells to induce tolerance in B cells, but also to identify their possible facultative role in this process.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA and (CBA × C57BL/6)F_1 mice weighing 18-20 g. Tolerance to SRBC was obtained by intraperitoneal injection of SRBC (6.2×10^9) and, 42-45 h later, of cyclophosphamide (CP), in a dose of 200 mg/kg [3]. Control animals received one of the above ingredients.

To study the immunologic competence of T and B lymphocytes of tolerant animals *in vivo*, suspensions of syngeneic thymus cells (5×10^{-7}-10×10^7), bone marrow cells (2×10^{-7}-5×10^7) or a mixture of both were injected intravenously into experimental mice 24 h after injection of CP. Six hours after transplantation of the cells the animals were given an intravenous injection of SRBC (5×10^8), and on the fourth day the number of antibody-forming cells (AFC) in the spleen was counted by Jerne's method.

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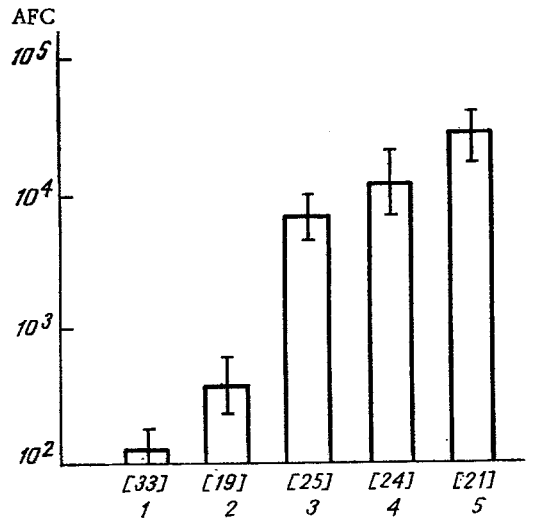

Fig. 1

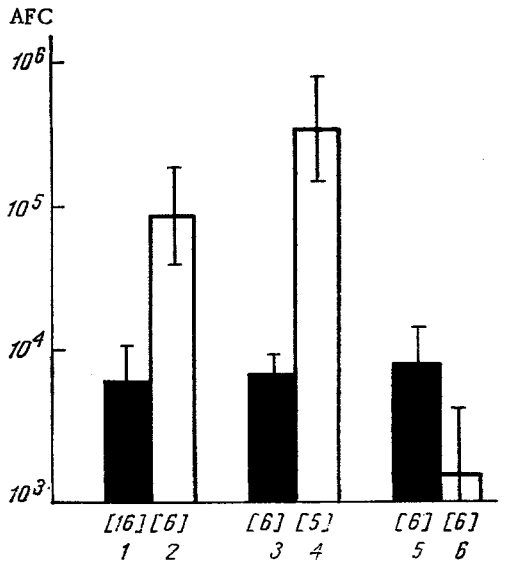

Fig. 2

Fig. 1. Immunologic activity of T and B lymphocytes of tolerant animals *in situ*. Abscissa, number of animals (in parentheses); ordinate, number of AFC in spleen of tolerant mice (1), of tolerant mice receiving bone marrow cells (2), thymus cells (3), thymus and bone marrow cells (4); animals receiving CP (5).

Fig. 2. Effect of ATS on immunoreactivity of spleen cells. Abscissa, number of animals (in parentheses); ordinate, number of AFC in recipients' spleen. Unshaded columns denote spleen cells of intact mice, black columns spleen cells of B mice; 1, 2) untreated cells; 3, 4) cells incubated with normal rabbit serum; 5, 6) cells incubated with ATS.

The formation of tolerance of B cells in the absence of T lymphocytes was investigated in mice artifically deprived of T cells (B mice). For this purpose the animals were thymectomized surgically by electric suction under hexobarbital anesthesia (100 mg/kg, intraperitoneally), and 2 weeks later the mice were irradiated in a dose of 950 R from a cobalt source (EKU-50 apparatus); 4 h later, 5×10^6-10×10^6 liver cells from 17-19-day syngeneic embryos were injected intravenously. Nonthymectomized mice receiving 5×10^7 syngeneic thymus cells along with embryonic liver cells after irradiation (TB mice) and intact animals served as the controls. At the end of each experiment the thymectomized animals were tested for completeness of removal of the thymus. Mice with remains of the thymus were rejected.

The B and TB mice 1-1.5 months after irradiation were subjected to complete and incomplete tolerogenic treatment. The immunologic status of the splenic lymphocytes of the experimental animals was investigated in cell culture *in vivo* seven days after the injection of CP. Syngeneic irradiated (950 R) recipients received an intravenous injection of 5×10^7 spleen cells from the experimental mice either separately or in various combinations with thymus (5×10^7), bone marrow (1×10^7), and spleen (1×10^7) cells from intact donors. In experiments to study suppressor activity 5×10^7-9×10^7 spleen cells of TB mice were injected into recipients together with 1×10^7 intact spleen cells.

In some experiments the spleen cells were first incubated (at 37°C, 45 min) with rabbit-anti-T serum (ATS) obtained in the writers' laboratory by the method described previously [1]. In parallel tests the cells were treated with normal rabbit serum.

The cells were transplanted into recipients simultaneously with injection of 2×10^6 SRBC, and four days later the animals were given a further intraperitoneal injection of 5×10^8 SRBC. The mice were killed eight days after transplantation of the cells and the number of AFC in their spleen was determined by Jerne's method.

TABLE 1. Formation of Tolerance to SRBC in B and TB Mice

Group No.	Source of transplanted cells and their number: spleen, 5×10^7	Source of transplanted cells and their number: intact thymus, 5×10^7	Number of recipients	Number of AFC in spleen: M_{geom}	Number of AFC in spleen: confidence interval
1	B NORM	—	16	5 859	3 205÷10 718
2	B NORM	+	8	396 489	308 468÷509 085
3	B AG	—	10	2 280	823÷6 329
4	B CP	—	17	95	<196
5	B CP	+	14	21 740	7 638÷61 802
6	B TOL	—	17	25	<57
7	B TOL	+	19	1 310	897÷2 460
8	TB NORM	—	11	93 362	67 608÷129 122
9	TB AG	—	10	305 791	208 869÷446 813
10	TB CP	—	20	39 526	27 040÷57 810
11	TB CP	+	6	35 637	21 429÷59 293
12	TB TOL	—	19	31	<78
13	TB TOL	+	14	912	450÷1 849
14	—	+	21	26	15÷46
15	—	+†	18	3 526	637÷16 672

*Combined with 10^7 intact bone marrow cells

Legend. TOL) Tolerant mice; AG) mice receiving 6.2×10^9 SRBC; CP) mice receiving 200 mg/kg CP; NORM) mice receiving no treatment

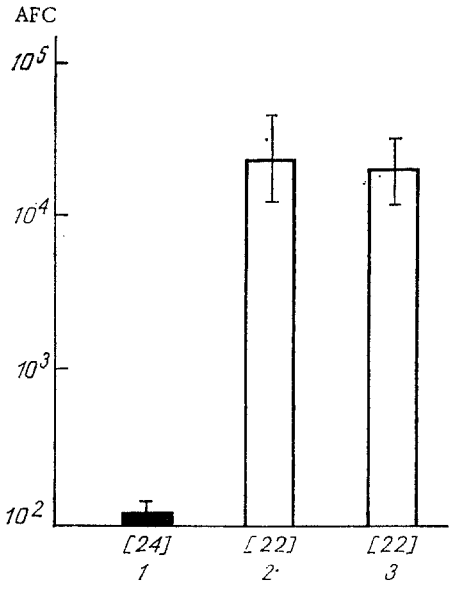

Fig. 3. Absence of suppressor activity of spleen cells of tolerant TB mice. Abscissa, number of animals (in parentheses); ordinate, number of AFC in recipients' spleen after injection of spleen cells of tolerant TB mice (1), of intact spleen cells (2), and of spleen cells of tolerant B mice together with intact spleen cells (3).

EXPERIMENTAL RESULTS

The results of investigation of the immunologic status of the T and B lymphocytes of tolerant animals are illustrated in Fig. 1. Experiments were carried out on 122 mice. As Fig. 1 columns 3, 4 show, thymocytes alone or mixed with bone marrow cells partially restored the immunoreactivity of the tolerant mice. After injection of bone marrow cells (Fig. 1 column 2) an effect also was observed, but it was weaker. The results indicate that in this form of tolerance the immunoreactivity of both T and B cells is specifically weakened, but injury to the T cells is dominant. This conclusion is in agreement with the writers' previous findings [3].

In the next series of experiments the possibility of induction of immunologic tolerance of B cells to SRBC was studied in the absence of T lymphocytes. The ability of the spleen cells of B mice, subjected to either complete (SRBC + CP) or incomplete (SRBC or CP) tolerogenic treatment, to cooperate with intact thymocytes was studied. The control consisted

of TB mice. As Table 1 shows, spleen cells of B mice either did not form AFC (groups 4 and 6) or formed only 1/20-1/100 (groups 1 and 3) of the number formed by cells of TB mice (groups 8, 9, and 10). On the addition of thymocytes to spleen cells of intact B mice (group 2) or of B mice receiving CP (group 5), the immune response was restored to its level observed in the control TB mice (groups 8-11). Some enhancement of the immune response also was observed if thymocytes were added to cells of tolerant B spleens (group 7) or tolerant TB spleens (group 13), but it was only 1/15-1/40 as strong as in the case of cooperation between thymocytes and spleen cells of B or TB mice receiving CP alone (groups 5 and 11). Specific tolerogenic treatment of B mice thus led to areactivity of B cells, just as occurred in TB mice receiving the same treatment.

The absence of T helpers in B mice was further confirmed in experiments in which spleen cells of the experimental animals were incubated with ATS: Such treatment (Fig. 2) considerably weakened the immunologic powers of the intact spleen cells, whereas B spleen cells produced the same number of AFC (relatively few) after incubation both with ATS and with normal serum. Some B cells are known to be able to react to thymus-dependent antigens even without cooperation with T cells [7].

Considering the observations of Gershon and Kondo [9] that T suppressors appear during tolerogenic treatment of TB mice, an attempt was made to find them in the case of tolerance induced with CP. As Fig. 3 shows, spleen cells of TB mice subjected to tolerogenic treatment had no suppressive effect on spleen cells of intact donors even though the former outnumbered the latter 5 or 9 times. In this form of tolerance, T suppressors were thus not activated. This conclusion agrees with observations of other workers [3, 5], although data to the contrary are also to be found in the literature [11].

The results thus show that participation of T helpers and of T suppressors is not essential for the formation of tolerance in a population of B cells, induced by thymus-dependent antigen with the aid of CP. No modifying effect of T cells on this process likewise was observed. This form of tolerance is evidently due to direct elimination of a definite clone of immunocompetent cells, induced by the antigen to proliferate, by the immunodepressant. This conclusion is in agreement with data on the character of action of CP on lymphocytes [4, 6] and on the conditions for induction of tolerance with the aid of CP [2].

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