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EFFECT OF SPONTANEOUS LOSS OF TOLERANCE ON T AND B LYMPHOCYTE FUNCTION IN MICE

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Functional activity of spleen and thymus cells of mice tolerant to sheep's red blood cells was studied 1 and 4 days after induction of tolerance. Tolerance was obtained with the aid of cyclophosphamide. Complete restoration of the immunocompetence of the thymus cells was found after 4 weeks. The functional activity of splenic T and B lymphocytes also was partly restored 4 weeks after induction of tolerance. Preliminary thymectomy weakened but did not prevent complete restoration of competence of splenic T cells. No T suppressors were found in the thymus and spleen of the tolerant animals.

KEY WORDS: immunologic tolerance; T and B lymphocytes; T suppressors; thymectomy.

In the absence of an antigen immunologic tolerance is spontaneously lost with the passage of time. In some forms of tolerance which has received the most study, function of the B cells is restored first, whereas the helper function of the T cells remains inhibited for several months [8]. Meanwhile, in tolerance induced by thymus-dependent antigens with the aid of cyclophosphamide (CP), partial recovery of the immunoreactivity of the lymphocytes in situ or in an adopted system was observed within 3 or 4 weeks [5, 7].

The object of the present investigation was to study the functional status of the B cells, T helpers, and T suppressors of the spleen and thymus in the stages of formation and partial loss of tolerance to sheep's red blood cells (SRBC), induced with the aid of CP. Because of the contradictory nature of data in the literature on the effect of thymectomy on formation and loss of tolerance [2, 7, 9, 12-14] it was decided to study the function of T and B lymphocytes in thymectomized animals subjected to the above-mentioned tolerogenic treatment.

EXPERIMENTAL METHOD

Male CBA and (CBA × C57BL/6)F_1 mice weighing 18-22 g were used. Tolerance to SRBC was induced by intraperitoneal injection of SRBC ($6.2 \cdot 10^9$) and CP (200 mg/kg). Control animals either received CP alone or were untreated. Tolerance was investigated 1 and 4 days after injection of CP. Thymectomy was performed on the animals 2 weeks before induction of tolerance. The donors' cells were injected intravenously into syngeneic recipients previously irradiated in a dose of 950 R (separately or in various combinations), in doses of $5 \cdot 10^7$ thymus and spleen cells of the experimental mice, $1 \cdot 10^7$ to $5 \cdot 10^7$ intact bone marrow cells, and $1 \cdot 10^7$ intact spleen cells. Simultaneously with the cells the animals received $2 \cdot 10^6$ SRBC, and 4 days later a

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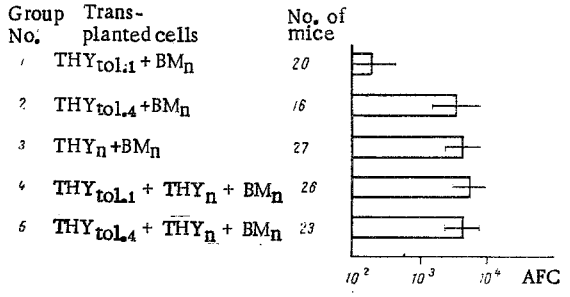

Fig. 1. Immunologic activity of thymus cells of tolerant animals at various times of investigation after tolerogenic treatment. THY$_{tol.1}$, THY$_{tol.4}$) Thymocytes of tolerant mice 1 and 4 weeks respectively after induction of tolerance; THY$_n$, BM$_n$) thymus and bone marrow cells of intact donors respectively. Here and in Fig. 2: abscissa, number of AFC in recipients' spleen.

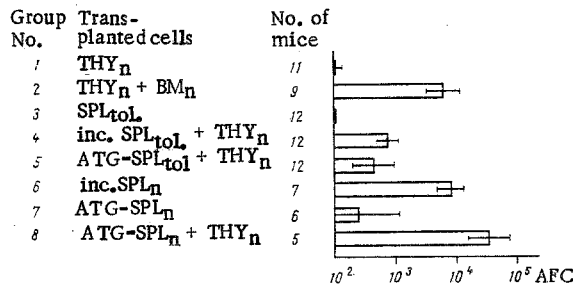

Fig. 2. Effect of ATG on cooperative activity of spleen cells of tolerant and intact animals. THY$_n$, BM$_n$, SPL$_n$) Thymus, bone marrow, and spleen cells respectively of intact donors; SPL$_{tol}$) spleen cells of tolerant donors 1 week after tolerogenic treatment; inc.SPL, ATG-SPL) spleen cells incubated with normal rabbit serum or with ATG respectively.

further intraperitoneal injection of $5 \cdot 10^8$ SRBC. On the 8th day after transplantation the animals were killed and the number of antibody-forming cells (AFC) in the spleen was counted by Jerne's method.

In some experiments the spleen cells were preincubated at 37°C for 45 min with rabbit anti-T globulin (ATG), obtained in the writers' laboratory by the method described in [3]. In parallel tests the cells were treated with normal rabbit serum.

EXPERIMENTAL RESULTS

In the experiments of series I the dynamics of helper activity of thymocytes of tolerant animals was studied in a cooperative test 1 and 4 weeks after tolerogenic treatment. The results of these experiments are summarized in Fig. 1. They show that 1 week after induction of tolerance the thymocytes were unable to cooperate with intact bone marrow cells (group 1), in good agreement with previous results showing injury predominantly to T helpers in this form of tolerance [4, 6, 10]. The level of the immune response after 4 weeks during cooperation between thymocytes of the experimental mice and intact bone marrow cells (group 2) corresponded to that observed during cooperation of intact lymphocytes (group 3). This indicated restoration of the helper activity of the thymocytes 1 month after induction of tolerance. The thymocytes of the tolerant animals, when investigated 1-4 weeks later, did not suppress cooperation of intact T and B cells (groups 4 and 5), which proved that the thymocytes studied had no suppressor activity.

The results of the next series (II) of experiments, devoted to the study of restoration of immunocompetence of splenic T and B lymphocytes of thymectomized tolerant animals are given in Table 1. Just as in the

TABLE 1. Dynamics of Restoration of Immunocompetence of T and B Lymphocytes in Thymectomized Tolerant Animals

Group No.	Time of testing, weeks	Transplantation of cells (number): spleens of various donors ($5 \cdot 10^7$)	Transplantation of cells (number): intact thymus ($5 \cdot 10^7$)	Transplantation of cells (number): intact bone marrow ($1 \cdot 10^7$)	Number of recipients	Number of AFC in spleen: M_g	Number of AFC in spleen: confidence intervals
1	1	te tol	—	—	23	14	<41
2		te tol	+	—	22	2 560	1 475—4 436
3		te tol	—	+	15	566	335—955
4		tol	—	—	13	45	<82
5		tol	+	—	21	1 094	690—1 735
6		tol	—	+	8	340	194—596
7		te cp	—	—	12	27 478	14 510—52 039
8		cp	—	—	7	31 806	22 276—45 307
9	4	te tol	—	—	22	10 277	5 084—20 785
10		te tol	+	—	10	93 321	51 808—168 115
11		te tol	—	+	5	17 582	8 985—34 394
12		tol	—	—	30	55 849	34 547—90 280
13		tol	+	—	9	104 765	72 207—151 852
14		tol	—	+	8	50 232	27 297—92 444
15		te cp	—	—	19	334 908	234 167—479 153
16		cp	—	—	14	270 557	110 459—661 912
17		—	+	—	33	26	<52
18		—	—	+	18	22	<113
19		—	+	+	37	11 856	7 718—18 217
20		normal	—	—	6	127 103	83 952—192 296

Legend: te) thymectomized mice; normal) intact mice; cp) mice receiving 200 mg/kg CP; tol) tolerant mice.

experiments of series I, the experiments were carried out 1 and 4 weeks after tolerogenic treatment. It will be clear from Table 1 that at the first time of testing (1 week) both the thymectomized (group 1) and the nonthymectomized animals (group 4) were equally well amenable to induction of tolerance, and the spleen cells of these animals were unable to form antibodies. In both cases their immunoreactivity was largely restored on the addition of intact thymocytes (groups 2 and 5), whereas intact bone marrow cells acted less effectively (groups 3 and 6). This indicated the formation of tolerance of the T cell type. Incidentally, the level of the immune response during cooperation between lymphocytes of thymectomized tolerant mice and thymocytes of intact donors (group 2) was significantly higher than in nonthymectomized tolerant animals (group 5).

During tests carried out after 4 weeks (Table 1) the splenic lymphocytes of both thymectomized (group 9) and nonthymectomized (group 12) tolerant donors restored ability to form antibodies, evidence that the T lymphocytes had spontaneously emerged from the state of nonresponsiveness and that their helper function was restored. The addition of intact thymocytes to spleen cells of thymectomized (group 10) and nonthymectomized (group 13) tolerant donors brought the immune response to the same level, possibly because of the identical maturity of the B cells of the two groups of animals. A different picture was observed on the addition of bone marrow cells of intact donors to the test spleen cells. In both cases (groups 11 and 14) the immunologic activity of the lymphocytes was virtually unchanged.

In the experiments of series III the action of ATG on immunoreactivity of splenic lymphocytes of tolerant and intact donors was studied in a cooperative test 1 week after induction of tolerance. The results of these experiments are given in Fig. 2. They show that ATG suppressed the immunologic powers of the T lymphocytes of the intact spleen (compare groups 7 and 6, 2, and 8). Incubation with ATG did not change the immunoreactivity of the splenic lymphocytes of tolerant animals, and during cooperation with thymocytes of intact donors (group 5) they produced the same number of AFC as after treatment with normal rabbit serum (group 4). However, the level of the immune response in both cases (groups 4 and 5) remained much lower than the control (groups 2, 6, and 8). The fact that no increase in the intensity of the immune response was observed in group 5 compared with group 4 confirmed the absence of activation of T suppressors in this form of tolerance.

It can be concluded from these results that during spontaneous loss of tolerance the helper function of the T cells of thymus and spleen and also the specific immunoreactivity of the splenic B cells are restored. The process of restoration of function of the T cells is significantly retarded by preceding thymectomy. No

sign of participation of T suppressors in the formation or maintenance of tolerance could be found. These conclusions are in agreement with experimental data obtained previously on the nature of this particular form of tolerance [1, 2, 4-6, 10].

Some unsolved problems must receive special attention. In particular, the reasons for the more effective cooperation of intact thymocytes with splenic B cells of thymectomized donors 1 week after induction of tolerance (Table 1, group 2) compared with the B cells of tolerant nonthymectomized mice (group 5), is not yet known. In the latter stages of the investigation this difference disappeared (Table 1, groups 10 and 13). The causes of the effect of thymectomy on the process of formation of tolerance in the population of B cells has not yet been explained.

Another problem arising during the analysis of the experimental results is that of the causes of partial restoration of immunoreactivity of the spleen cells of thymectomized tolerant animals (Table 1, groups 9 and 1). This restoration of immunoreactivity cannot be ascribed to an increase in the number of immunocompetent B lymphocytes as tolerance is lost (Table 1, groups 9 and 3).

Partial restoration of the helper function of the lymphocytes in the absence of the thymus can be explained either by the formation of new clones of immature T cells capable of functioning as helpers [11] or by the reversible character of tolerogenic inactivation of the T cells [1] or, finally, by proliferation of T cells left undamaged after tolerogenic treatment. It is too early as yet to decide which of these possible explanations is to be preferred.

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