EFFECT OF THYMECTOMY ON INDUCTION OF TOLERANCE AND ON T SUPPRESSOR FORMATION

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During the last 10 years the nature of immunologic tolerance has been extensively discussed in the literature. It has not been shown that tolerance may be based on two fundamentally different processes: elimination or inactivation by an antigen of the corresponding clone of immunocompetent cells [6, 9, 10, 16] or increased activity of antigen-specific T or B suppressors, secondarily inhibiting effector cells or their precursors [8, 14, 15]. The concrete conditions under which a particular form of tolerance arises and the possibility of their coexistence and mutual transition are items for discussion [3].

The writers showed previously that during tolerance to sheep's red blood cells (SRBC) obtained in adult animals with the aid of immunodepressant cyclophosphamide, successive activation of the two mechanisms is possible. The primary mechanism of this form of tolerance is evidently clonal deletion, but in the later stages suppressor cells which may play a role in the maintenance of tolerance appear [4].

The object of the present investigation was to study the nature of suppressor cells in tolerant animals and also to investigate the possibility of their appearance in animals previously thymectomized.

EXPERIMENTAL METHOD

Adult (CBA × C57BL/6) mice were obtained from the Nursery of the Academy of Medical Sciences of the USSR. Some of the mice were subjected to preliminary thymectomy (2-3 weeks before the experiment) by Miller's method [11], with removal of the lobes of the thymus by means of an electric suction pump through an incision of the upper third of the sternum.

To induce tolerance in thymectomized and also in nonthymectomized animals, 6×10^9 SRBC and cyclophosphamide (CP) in a dose of 200 mg/kg body weight were injected intraperitoneally, one after the other, with an interval of 2 days between injections. After 14 days immunoreactivity and suppressor activity of the spleen cells of the experimental and control animals were tested in an adoptive system. For this purpose, spleen cells of thymectomized and nonthymectomized tolerant animals (8×10^7) were injected intravenously, separately or together with cells from intact donors (1×10^7), in combination with 2×10^6 SRBC, into syngeneic recipients irradiated in a dose of 950 R. After 4 days the recipients were given a further intraperitoneal injection of 5×10^8 SRBC, and 4 days later still the number of antibody-forming cells (AFC) in the recipients' spleen was determined by Jerne's method.

In some experiments the spleens of tolerant donors were treated with rabbit anti-T-globulin (ATG) [1]. The treatment for 45 min at 37°C in the presence of fresh rabbit's complement in the production of 1×10^8 cells, 0.25 ml ATG, and 0.5 ml complement in medium No. 199 with the addition of 5% embryonic calf serum and HEPES buffer. The final cell concentration was 2×10^7 in 1 ml medium and the final dilutions of ATG and complement were 1:20 and 1:10 respectively. In control tests the cells were treated with normal rabbit serum (NRS) under the same conditions. After incubation and washing twice the control and experimental suspensions were studied in an adoptive system by the method described above.

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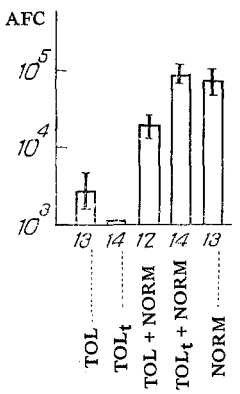

Fig. 1

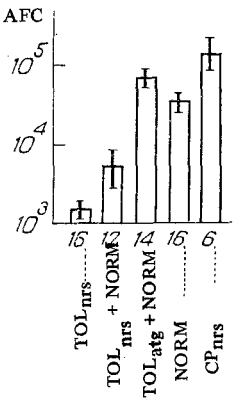

Fig. 2

Fig. 1. Effect of thymectomy on tolerance formation and on suppressor activity of lymphocytes. TOL) Tolerant nonthymectomized donors; TOL_t) tolerant thymectomized donors; NORM) intact donors. Abscissa, above — number of recipients, below — donors; ordinate, number of AFC in recipients' spleen.

Fig. 2. Effect of ATG on suppression of immune response of intact lymphocytes by spleen cells of 14-days tolerant animals. TOL_{nrs}) Spleen cells of tolerant donors incubated with NRS; TOL_{atg}) spleen cells of tolerant donors incubated with ATG; CP_{nrs}) spleen cells of donors receiving CP, incubated with NRS; NORM) cells of intact donors. Abscissa: above — number of recipients, below — cells injected; ordinate, number of AFC in recipients' spleen.

During statistical analysis of the experimental data the geometric mean, the error of the means, and confidence intervals were calculated by Student's method.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of thymectomy on tolerance formation and on the suppressor activity of the lymphocytes was studied. The results of a comparative study of spleen cells of tolerant thymectomized and nonthymectomized animals in an adoptive system are given in Fig. 1. They show that after thymectomy and treatment of the animals with the tolerogen the level of the immune response of the spleen cells in the adoptive system was significantly lower than the response of the spleen cells of nonthymectomized animals. Combined culture of lymphocytes of tolerant animals and of intact donors led to suppression of the immune response of the latter. If, however, the tolerant donors were thymectomized beforehand, the suppressor effect was absent.

Preliminary thymectomy thus led to deepening of tolerance and, at the same time, prevented the formation of suppressor cells. The higher intensity and longer duration of tolerance in thymectomized animals were probably linked with disturbance of the restoration of functions of the T helpers after thymectomy. These results are evidence of definite dissociation between tolerance formation and suppressor cell formation and they indicate that tolerance can be obtained even in the absence of suppressors. This conclusion is confirmed by previous observations showing that tolerance can be induced in a pure population of B cells in B mice in the complete absence of T cells, including T suppressors [2].

The fact that suppressor cells are absent in thymectomized animals suggests that they are T cells. To clarify the nature of the suppressor cells special experiments were carried out with rabbit anti-T-antibodies. Tolerance was induced in mice by the standard method.

After 14 days their spleen cells were treated with ATG in the presence of complement as described above. The control suspension of cells from tolerant animals was incubated under the same conditions with complement alone. The cells treated in this manner were injected into irradiated recipients either separately or together with intact lymphocytes. The results are given in Fig. 2. They show that spleen cells of tolerant animals incubated without ATG, just as in the previous experiments, significantly inhibited the immune response of intact lymphocytes. Treatment of cells of tolerant donors with ATG in the presence of complement completely abolished the inhibitory effect. The sensitivity of the suppressor cells to the cytotoxic action of anti-T-antibodies is evidence of their T-cell nature.

On the whole the results confirm the previous hypothesis [4] that the mechanism of immunologic tolerance obtained in adult animals by combined injections of a thymus-dependent antigen (SRBC) and the immunodepressant cyclophosphamide is based on two processes: elimination or irreversible inactivation of the corresponding clone of immunocompetent cells and activation of antigen-specific T suppressors. The dominant factor is clonal deletion. It can be detected in the earliest stages of tolerance formation and may determine the long persistence of tolerance if new clones of T cells are not formed, as is the case in thymectomized animals. Under ordinary conditions tolerance is maintained by T suppressors, which appear in the later stages. The probable mechanism of suppressor cell formation may be activation of their precursors by persistent antigen against the background of elimination of the cell clone that reacts positively to the antigen by the cytostatic. The later appearance of T suppressors is evidently linked with their high sensitivity to CP [5, 7, 13].

A combination of clonal-deletion and suppressor mechanisms, with dominance of the former, has been established in recent years in several other forms of tolerance also [9, 10, 12]. This situation suggests that the principles established in the experimental model described in this paper may be more general in character.

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