

EFFECT OF THYMECTOMY ON INDUCTION OF IMMUNOLOGIC TOLERANCE IN ADULT MICE AND ON RESTORATION OF ORIGINAL IMMUNOREACTIVITY

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Removal of the thymus strengthened and prolonged tolerance to sheep's red blood cells obtained with the aid of cyclophosphamide if thymectomy was performed on the mice before or after the induction of tolerance. Thymectomy had its greatest effect if performed 24 h before the induction of tolerance. The results confirm the view that this form of tolerance is due to a deficiency of a definite clone of T helpers.

KEY WORDS: immunologic tolerance; cyclophosphamide; thymectomy; T lymphocytes.

Several concepts have been suggested in order to explain the mechanism of immunologic tolerance. According to one of them, the cause of specific tolerance is the elimination or inactivation of the corresponding clone of immunocompetent cells (for thymus-dependent antigens these are the T helpers [1, 4, 13]). Other workers explain the state of immunologic tolerance by activation of T suppressors [3, 6]. To study this problem various factors have been used to inhibit the function of the T cells, such as thymectomy, administration of antilymphocytic serum, and so on [7]. According to earlier studies [2, 5, 12], preliminary thymectomy deepens and prolongs the state of tolerance to certain thymus-dependent antigens. In recent years, however, new data not confirming this hypothesis have been obtained [8, 10, 11].

The object of this investigation was to study the effect of thymectomy on the formation of tolerance to sheep's red blood cells (SRBC) obtained with the aid of cyclophosphamide (CP) and on the dynamics of the subsequent recovery of immunoreactivity.

EXPERIMENTAL METHOD

Noninbred male mice and (CBA \times C57BL/6) F_1 male mice, weighing 18–20 g, were used. Tolerance to SRBC was induced by intraperitoneal injection of $6.2 \cdot 10^9$ SRBC and, 42–45 h later, intraperitoneal injection of CP in a dose of 200 mg/kg [1]. Animals receiving CP and intact mice served as the control.

In series I some of the experimental and control animals were thymectomized 2 weeks before induction of tolerance. The operation was performed under hexobarbital anesthesia (100 mg/kg, intraperitoneally), using a surgical electric suction apparatus. At various times after induction of tolerance (7, 30, and 90 days) the animals were given an intravenous injection of $5 \cdot 10^8$ SRBC, and the number of antibody-forming cells (AFC) in the spleen was counted on the 4th day by the method of local hemolysis in gel.

In the experiments of series II thymectomy was performed before the induction of tolerance (1 or 14 days before injection of SRBC) or after induction (3 h after injection of CP). Animals undergoing mock thymectomy served as an additional control. One month after the injection of CP the mice were given an intravenous injection of $5 \cdot 10^8$ SRBC; the number of AFC in the spleen was counted on the 4th day by Jerne's method.

In all the experiments the completeness of removal of the thymus was verified before determination of the number of AFC in the thymectomized animals. Mice with remains of the thymus were rejected.

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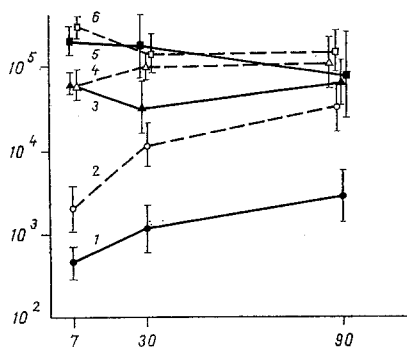


Fig. 1

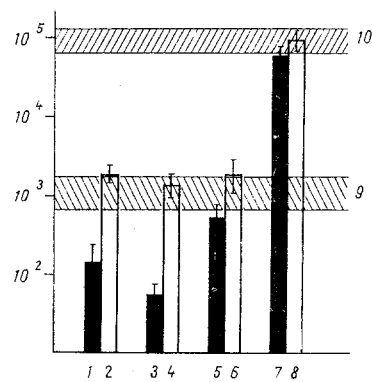


Fig. 2

Fig. 1. Dynamics of recovery of immunoreactivity in tolerant and control animals. 1) Thymectomized tolerant mice; 2) tolerant mice; 3) thymectomized mice receiving CP; 4) mice receiving CP; 5) thymectomized mice; 6) intact mice. Ordinate, number of AFC in spleen; abscissa, time of investigation, days.

Fig. 2. Effect of interval between operation and induction of tolerance on level of tolerance. Black columns denote thymectomized mice; unshaded columns mice undergoing mock thymectomy. 1, 2) Operation 14 days before induction of tolerance; 3, 4) operation 1 day before induction of tolerance; 5, 6) operation 3 h after induction of tolerance; 7, 8) operation 16 days before injection of CP; 9) level of immune response of tolerant animals; 10) level of immune response of mice receiving CP. Ordinate, number of AFC in spleen.

EXPERIMENTAL RESULTS

The results of investigation of the formation and dynamics of recovery of immunoreactivity in the tolerant and control, thymectomized and intact, animals are shown in Fig. 1. Experiments were carried out on 310 noninbred mice. Tolerance was studied on the 7th, 30th, and 90th days after injection of CP. As Fig. 1 shows, thymectomy increased tolerance starting from the first time of investigation. Later during the experiments the differences between the thymectomized and nonthymectomized tolerant mice increased, evidence of disturbance of the recovery of immunoreactivity as a result of thymectomy. The deepening and prolongation of tolerance in the thymectomized animals were in agreement with evidence obtained previously that the main factor in this form of tolerance is a deficiency of T cells [1]. Nevertheless, as is clear from Fig. 1, ability to form antibodies in thymectomized tolerant animals nevertheless increased almost sixfold in the course of time. Partial recovery of immunocompetence, despite the thymectomy, can be explained in two ways: 1) blockade of T helpers in this form of tolerance may be reversible; 2) after thymectomy natural death of the T suppressors is observed [8, 9], leading to intensification of the immune response. The second suggestion is contradicted by the fact that thymectomy in the control animals had no significant effect on the intensity of the immune response regardless of the time of observation (Fig. 1).

The results of experiments to compare the effectiveness of induction of tolerance depending on the time of thymectomy are summarized in Fig. 2. Experiments were carried out on 104 male (CBA \times C57BL/6) F_1 mice weighing 18-20 g. As will be clear from Fig. 2, removal of the thymus in this series of experiments also intensified tolerance whatever the time of thymectomy. The operative trauma itself did not affect the formation of tolerance: In tolerant animals undergoing mock thymectomy (groups 2, 4, 6) the AFC level agreed exactly with the AFC level in intact tolerant animals (groups 1 and 3). If, however, the mice were thymectomized immediately after the induction of tolerance, this operation was reflected in the level of tolerance much less strongly (group 5). These differences perhaps indicate that during the induction of tolerance some precursors of T helpers avoid inactivation by migrating from the thymus into the peripheral lymphoid organ. It was shown previously [1] that during the induction of tolerance intrathymic and extrathymic precursors of T helpers are inactivated equally. However, the intermediate stage of differentiation of the T helpers is evidently more resistant to this process.

It follows from Fig. 2 that tolerance in animals thymectomized beforehand was weaker if the operation was performed 1 day, and not 14 days, before injection of the antigen (groups 1 and 3). In that case the long-term consequences of thymectomy were evidently apparent - a change in the ratio between individual subpopulations of T cells in the tolerant animals, marked exhaustion of short-living T_1 cells [8, 8], and loss of control over their differentiation by thymus hormones.

The results confirming the effect of thymectomy on the recovery of immunoreactivity in tolerant mice are in harmony with the observations of Aisenberg et al. [2]. Contrary data were obtained on other models of tolerance (injection of de-aggregated protein antigens [9, 11]). The time chosen by the present writer for the operation (2 weeks before injection of the antigen) led to reduction of the T suppressors by the time of induction of tolerance [8, 9]. The fact that thymectomy not only did not prevent the induction of tolerance, but actually intensified it, points to the clonal-deficiency nature of this form of areactivity. Meanwhile, the more marked intensification of tolerance when thymectomy was performed 1 day and not 14 days beforehand can be interpreted as evidence of the participation of short-lived T suppressors in the induction of tolerance. However, participation of T suppressors is not an essential component of this process.

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