# INDUCTION OF IMMUNOLOGIC TOLERANCE IN ADULT MICE BY REPEATED INJECTION OF ALLOGENIC SPLEEN CELLS

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Adult C57BL mice were repeatedly immunized with viable spleen cells from C3H mice. Production of antibodies to foreign spleen cells stopped in the majority of animals and a state of immunologic tolerance arose. Spleen cells of tolerant C57BL mice did not evoke a systemic transfer reaction in C3H mice.

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We have previously shown [2] that repeated injection of xenogenic spleen cells of C3H mice into Wistar rats may produce a state of immunologic tolerance to the injected antigen in these animals.

In the present investigation the possibility of creating immunologic tolerance was studied in mice differing in their strong tissue-compatability H-2 locus, by giving repeated injections of foreign spleen cells over a long period of time.

## EXPERIMENTAL METHOD

Forty C57BL/6 mice aged 1 month and weighing initially 15 g were repeatedly immunized with a freshly prepared suspension of spleen cells of C3H/He mice. Immunization was carried out intraperitoneally with 0.2 ml of suspension (20 million viable nucleated cells) three times a week at intervals of 2-3 days. The methods of preparations of the suspension and determining the number of viable cells in them by means of a 0.1% solution of trypan blue have been described earlier [3].

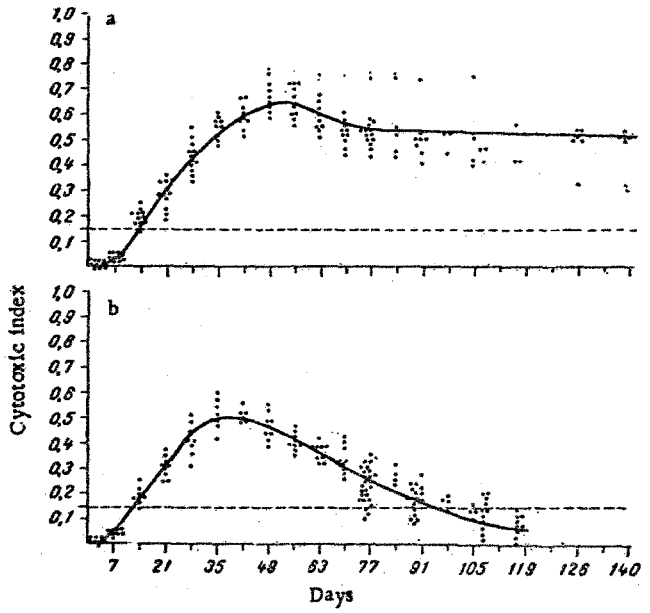


Fig. 1. Dynamics of cytotoxic isoantibodies in C57BL mice receiving repeated injections of spleen cells of C3H mice. a) Immune type of response, b) response of development of tolerance.

The state of immunologic activity of the C57BL/6 mice was tested from the dynamics of cytotoxic isoantibodies, determined in vitro by the method of Gorer and O'Gorman [6], with a modification to allow for the fact that the sensitivity of the cytotoxic reaction increases with the use of smaller doses of target cells [1].

The target consisted of nucleated spleen cells of C3H mice. Antiserum was investigated on the 3rd and 7th days from the beginning of the immunization cycle, and thereafter at weekly intervals. The cytotoxic index of antiserum diluted 1:5 was determined by the usual method [7]. The experimental animal was considered to be tolerant if the cytotoxic index of the serum was below 0.15.

The degree of sensitization of the spleen cells of tolerant and immune C57BL mice was determined by the systemic transfer reaction method [5]. C3H mice were injected intra-

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TABLE 1. Results of Systemic Transfer Reaction in C3H Mice

| Donors of transferred spleen cells | C3H Mice | |
|---|---|---|
| | Number | Splenic index (M ± Ip) |
| Intact C57BL | 22 | 1 |
| C57BL sensitized once | 20 | 1.842 ± 0.137 |
| C57BL sensitized repeatedly | 12 | 2.309 ± 0.146 |
| Tolerant C57BL | 12 | 0.976 ± 0.031 |
| Isologus (C3H) | 20 | 0.961 ± 0.038 |

peritoneally with 80 million viable nucleated spleen cells from tolerant or sensitized (60 or 1 injection of spleen cells 7 days before transfer) C57BL mice. Control C3H mice received analogous injections of spleen cells from intact C57BL or C3H mice. Five days after transfer of the cells from these groups of animals the reaction was read by taking note of the splenic index (the index in C3H mice receiving injections of spleen cells of intact C57-BL mice was taken as unity). The numerical results were analyzed by statistical methods and the arithmetical mean (M) and confidence interval (Ip) calculated with a probability of 99%.

## EXPERIMENTAL RESULTS

Cytotoxic isoantibodies were detected in the C57BL mice after the 14th day of the experiment (six injections of antigen). Two types of reaction to repeated injections of allogenic spleen cells were observed (Fig. 1). Of the 40 experimental mice 6 died on the 30th-50th day, and of the 34 survivers, 12 (35%) gave a reaction of immune type and 22 (65%) developed tolerance. In the mice giving an immune type of response to repeated injections of foreign spleen cells, cytotoxic isoantibodies were formed throughout the period of observation (140 days). In the animals developing tolerance, antibody formation stopped despite continued injections of antigen.

Cases were observed when tolerant mice died if injection of foreign viable spleen cells continued for more than two weeks after development of tolerance. The animals which died showed characteristic signs of established homologous disease. This phenomenon of death of adult mice following repeated injections of immunologically competent cells of a donor to which a state of tolerance has been established has been described by many investigators and is attributed to the occurrence of a "graft versus host" reaction in a situation when the host is areactive toward the injected cells [4]. No cases of death of mice immunologically active against the injected spleen cells were observed over a period of 50-140 days.

Estimation of the degree of sensitization of spleen cells of the immune and tolerant C57BL mice showed that splenomegaly developed in the C3H mice receiving injections of spleen cells from donors sensitized with tissues of the recipients (Table 1). In the case of transfer of spleen cells from repeatedly sensitized C57BL mice the splenomegaly was more marked than when cells were transferred from animals sensitized only once. If the same number of cells was injected from isologous or intact C57BL mice, no splenomegaly developed in the C3H mice on the 5th day after transfer. Intergroup differences in the magnitude of the splenic index when spleen cells from tolerant and sensitized mice were used were statistically significant ($P = 0.001$).

The spleen cells of mice in which a state of immunologic tolerance has been induced as a result of repeated injections of antigen thus do not evoke a systemic transfer reaction in the animals to whose tissues tolerance was induced.

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