# INDUCTION OF TOLERANCE TO ALLOANTIGENS IN ADULT MICE

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Tolerance to allogeneic C57BL/6 (H-$2^b$) cells was induced in CBA (H-$2^k$) mice. A combination of three types of intervention was used for tolerogenic treatment: thymectomy on adult mice, injection of a large dose of donor's spleen cells, and injection of 200 mg/kg cyclophosphamide 24 h later. Long (up to 5 months) survival of transplanted allogeneic C57BL/6 hearts was observed in animals treated in this way. Tolerance to antigens of the H-2 complex was confirmed in mixed lymphocyte culture. No chimerism was present in the recipients' lymphoid tissue.

KEY WORDS: immunologic tolerance; suppression of tissue incompatibility; transplantation of the heart; cyclophosphamide; thymectomy.

Long persistence of an antigen in the body leads to the development and maintenance of tolerance to that antigen [4]. In the case of transplantation antigens one method of maintaining tolerance is chimerism of hematopoietic and lymphoid tissues, which facilitates long persistence of the donor's antigens in the recipient. Such a situation can be achieved by combined treatment with immunodepressive agents, such as irradiation or chemotherapeutic substances, and by injection of a sufficiently high dose of hematopoietic or lymphoid cells. It has been shown, for instance, that injection of spleen cells of $F_1$ hybrid mice into recipients of a parental line a few hours after injection of the immunodepressant cyclophosphamide (CF) leads to long-lasting chimerism of the lymphoid tissue and promotes tolerance to skin grafts or transplantation of the heart of the donor line [2, 6, 10, 13].

However, it is difficult to obtain chimerism of lymphoid tissue in a completely allogeneic system, because of the development of a "graft versus host" reaction (GVHR) against the background of immunodepression of the recipient. The writers therefore have attempted to create tolerance to an allograft without the development of marked chimerism, using a combination of several immunodepressive procedures and a different order of their application. It was shown previously that specific elimination of a clone of stimulated CBA anti-C57BL/6 lymphocytes can be obtained under the influence of CP by injecting $1 \cdot 10^8$ C57BL/6 mouse spleen cells into CBA mice 24 h before injection of 200 mg/kg CP [8]. Thymectomy on adult mice is also known to prolong tolerance to thymus-dependent antigens [12, 15].

The object of this investigation was to test a new system of induction of tolerance by means of thymectomy and injection of allogeneic cells and CP.

## EXPERIMENTAL METHOD

Male and female CBA (H-$2^k$), C57BL/6 (H-$2^b$), BALB/c (H-$2^d$) and (CBA×C57BL/6)$F_1$ (H-$2^{kb}$) mice were used. Thymectomy was performed on adult CBA mice aged 2-3 months by the usual method [11]. Tolerogenic treatment was carried out 1-2 months by the usual method [11]. Tolerogenic treatment was carried out 1-2 months after thymectomy. It consisted of injection of $1 \cdot 10^8$ C57BL/6 spleen cells intravenously, followed by injection of CP in a dose of 200 mg/kg intraperitoneally 18-24 h later. Additionally, 3-6 h after CP, some of the animals received an injection of embryonic liver cells from C57BL/6 or (CBA×C57BL/6)$F_1$ mice in a dose of $2.5 \cdot 10^7$-$1 \cdot 10^8$ cells per mouse. The state of tolerance was assessed by heterotopic skin grafting from C57BL/6 mice [1] and transplantation of the heart of newborn mice of the same line subcutaneously beneath the skin of the ear of the experimental animals [6]. Survival of

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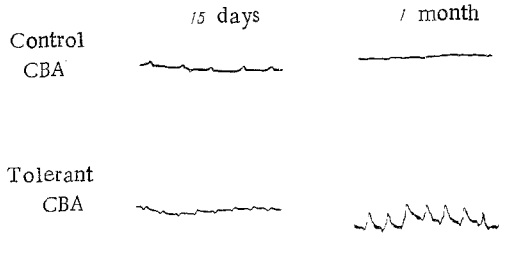


Fig. 1

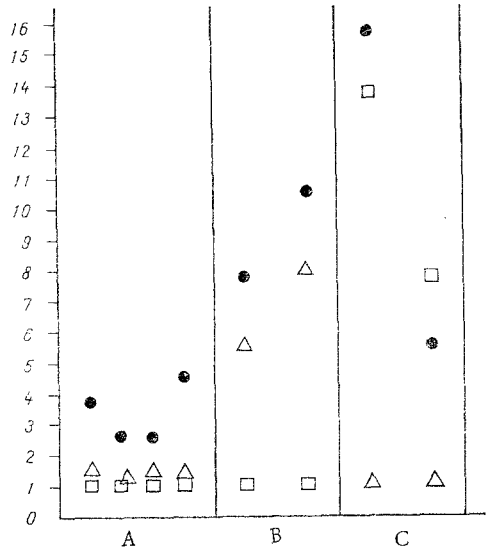


Fig. 2

Fig. 1. Electrical activity of heterotopic allogeneic heart grafts from C57BL/6 mice on CBA mice. Above: EDG of heterotopic allogeneic heart grafts on control mice on 15th day and 1 month (ECG absent) after transplantation. Below: ECG of heterotopic allogeneic heart grafts on CBA mice subjected to tolerogenic treatment. Positive ECG 1 month after transplantation.

Fig. 2. Reactivity of spleen cells of mice tolerant to alloantigens with respect to cells of different lines in MLC. Abscissa, responding cells: A) of tolerant mice (response of individual mice); B) of CBA mice; C) of C57BL/6 mice; ordinate, index of stimulation. For B and C results of two experiments are given, in which pulped cell suspensions were used. Response to mouse cells shown by appropriate symbols: squares — CBA; triangles — C57BL/6; filled circles — BALB/c.

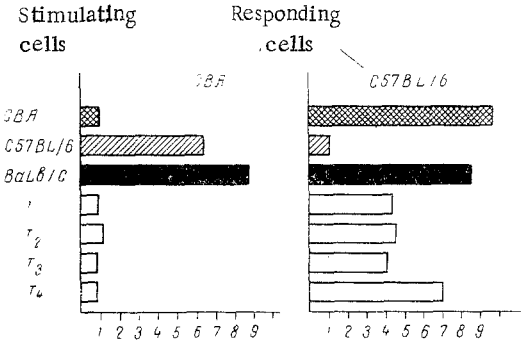


Fig. 3. Intensity of response in MLC of spleen cells of CBA and C57BL/6 mice to cells of tolerant and control mice. Abscissa, index of stimulation; ordinate, stimulating cells.

the skin graft was determined visually, whereas survival of the heart was assessed by recording electrical activity of the graft on the 1T-03M electrocardiograph. Tolerance of the recipient's antigen-recognizing cells to H-$2^b$ antigens (the haplotype of the C57BL/6 mice) was tested by determining the intensity of blast-transformation in a mixed lymphocyte culture (MLC) of tolerant mice with spleen cells from C57BL/6, BALB/c, and CBA mice. The presence of chimerism in tolerant mice also was investigated by the blast-transformation reaction in MLC; cells of tolerant mice were used as stimulating cells. The MLC were set up in the writers' own modification in siliconized penicillin flasks [7].

## EXPERIMENTAL RESULTS

In frequently repeated experiments carried out in accordance with the scheme described above (thymectomy — C57BL/6 cells — CP — C57BL/6 heart) a high percentage of survival of the allogeneic heart was observed, virtually no rejection occurring throughout the period of observation. For instance, of 38 mice into which an allogeneic heart was transplanted 20 days or more after tolerogenic treatment, a positive ECG was recorded in 33 animals for 24-30 days and in 12 of 18 mice for over 3 months. In some experiments the observations ceased after 1 month. In the control the ECG of the graft could no longer be recorded after 14-16 days (Fig. 1). It is interesting to note that the heart did not take if it was transplanted immediately or soon after tolerogenic treatment. Failure of tolerance also was observed in groups in which embryonic liver cells from C57BL/6 or (CB×V57BL/6)$F_1$ mice were injected after CP. During skin grafting which, in some experiments, was carried out on the same experimental animals but 16 days to 1 month after tolerogenic treatment and before transplantation of the heart, only a very small increase in the duration of survival of the allogeneic skin graft was observed.

Evaluation of the results of blast transformation in MLC showed that spleen cells of tolerant mice did not react to H-2 antigens of C57BL/6 mice although they continued to react to antigens of the third line — BALB/c (Fig. 2). Meanwhile, when cells of tolerant mice were used as stimulating cells, C57BL/6 lymphocytes reacted to them whereas CBA lymphocytes did not react (Fig. 3). Consequently, the spleen of the experimental animals contained C57BL/6 cells.

Tolerance to C57BL/6 ($H\text{-}2^b$) antigens was thus confirmed in MLC and it was shown that this tolerance was not due to chimerism of the experimental animals. The possibility cannot be ruled out that the cardiac allograft itself, as the source of antigen, played an essential role in the maintenance of tolerance.

In four CBA mice in which a positive ECG of the graft was recorded for over 5 months, attempts were made to induce rejection of the transplanted C57BL/6 heart by injection of, initially $3 \cdot 10^7$, and later $1 \cdot 10^8$ normal spleen cells of CBA mice. However, the graft was not rejected.

Prolonged tolerance to transplantation antigens was thus created in the presence of a complete difference between donors and recipients for the H-2 complex. The scheme of obtaining tolerance which was used was similar in principle to induction of tolerance against sheep's red blood cells (SRBC) or Vi-antigen [9, 3, 5]. In both cases the antigen was injected first (Vi-antigen, SRBC, and so on), and CP later. One difference of the suggested scheme of tolerogenic treatment is the use of preliminary thymectomy on adult mice. Tolerance to SRBC and Vi-antigen can be obtained with the aid of CP on nonthymectomized mice also. Tolerance to alloantigens in the presence of a complete difference for the H-2 complex could not be obtained by the present writers without thymectomy, although injection of allogeneic spleen cells 24 h before CP considerably improved and prolonged survival of semiallogeneic and allogeneic cells injected after CP [8]. If the thymus is preserved, tolerance is evidently quickly lost if not maintained by long-persisting donor's allogeneic cells. It can be tentatively suggested that the cause of the form of tolerance observed is elimination by CP of antigen-stimulated clones of T-cells [2-5, 8]. However, the possibility cannot be ruled out that long-living T-suppressors are present in mice tolerant to the allografted heart, as is shown indirectly by failure of attempts to overcome tolerance by injecting intact CBA spleen cells into the experimental mice.

The differences between the results of skin and heart transplantation can perhaps be attributable to the presence of Sk-antigens in the skin, but not in lymphocytes or in cells of the transplanted heart [14]. A further study is required both of the mechanism of induction of this form of tolerance and of the causes of the abolition of tolerance by allogeneic embryonic liver cells.

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# INDUCTION OF CYTOCHROME P-450 AND AN IMMUNE RESPONSE BY PHENOBARBITAL, FREE AND COVALENTLY BOUND WITH ALBUMIN

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Definite interaction exists between the induction of microsomal enzymes and induction of the immune response to foreign substances. The loss by phenobarbital of its ability to induce the cytochrome P-450-hydroxylase system when bound covalently with albumin, coupled with the acquisition by phenobarbital of ability to induce a lymphocytic immune system reflects the ability of the two defensive systems of the body — hydroxylase and immune — to interact with foreign compounds of low and high molecular weight. The cytochrome P-450-hydroxylase system of the liver and other tissues is designed to protect the body against the action of low-molecular-weight hydrophobic compounds. The immune system is responsible for the protective effect against high-molecular-weight foreign substances.

KEY WORDS: induction; phenobarbital; albumin; cytochrome P-450; immune response.

Many investigations have shown that administration of phenobarbital (PB) to animals leads to the induction of cytochrome P-450 in the liver, thereby stimulating reactions of hydroxylation both of PB itself and of many other foreign compounds [1]. Induction of the hydroxylase system is accompanied by synthesis of mRNA and enzyme proteins of membranes of the endoplasmic reticulum [7]. Meanwhile injection of PB bound covalently to a macromolecular carrier (a protein, for example), into animals leads to induction of synthesis of immunoglobulins specifically binding with PB and certain structurally related compounds, and inactivating them [3-6], in the lymphocytes. It has been suggested that definite interaction exists between the induction of microsomal enzymes and induction of the immune response to foreign substances. Low-molecular-weight foreign substances induce the hydroxylase systems of the liver, high-molecular-weight substances induce the immune system of the lymphocytes. According to this view, the hydroxylase and immune systems can be regarded as a single system of protection of the organism against the action of foreign substances of low and high molecular weight.

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