PRODUCTION OF IMMUNOLOGICAL TOLERANCE TO MICROBIAL AND CROSS-REACTING MOUSE TISSUE ANTIGENS

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By injecting *Candida albicans* vaccine into newborn CC57BR mice tolerance was obtained to the microbial antigen, a manifestation of which was inhibition of the rejection of skin grafts from C3H mice. In the tolerant animals the number of lymphocytes reacting with microbial and donor's tissue antigen in the immunoadhesion and blast-transformation reactions was reduced and their cytotoxic activity against fibroblasts of C3H mice was lowered. Tolerance to microbial and cross-reacting transplantation antigens was transferred by spleen cells to syngeneic recipients irradiated in a dose of 700 R.

KEY WORDS: immunological tolerance; cross-reacting antigens; skin grafting.

Several workers, on the basis of their results, have postulated the existence of antigens related to tissue-compatibility antigens in certain microorganisms (*Streptococcus*, *Candida albicans*) [1, 4, 6]. Sensitization of an animal by such cross-reacting microbial antigens induces acceleration of allograft rejection [1, 6] as a result of the development of an immunological reaction against the related transplantation antigens. However, the question of the possible use of cross-reacting microbial antigens in order to prolong the survival of allografts by producing immunological tolerance to them has not received due attention.

This paper describes a study of the possibility of obtaining immunological tolerance to antigens of *C. albicans* and to related mouse tissue antigens.

EXPERIMENTAL METHOD

The experimental animals were female mice weighing 16-18 g belonging to strain C3H ($H\text{-}2^k$) for the donors and CC57BR ($H\text{-}2^b$) for the recipients. Tolerance was induced in the CC57BR mice by intramuscular injections of heat-killed *C. albicans* vaccine daily for the first 3 days after birth and thereafter weekly for 3 weeks, in a dose of 10^{10} cells per injection. With this method of producing tolerance 40% of the animals died. The scheme of the experiments was as follows: 1 month after the last injection of antigen the experimental (tolerant) and control (intact animals of the same age) CC57BR mice were divided into five groups. Group 1, to test the state of tolerance, was immunized with four weekly intraperitoneal injections of 10^{10} *C. albicans* cells in 0.3 ml Freund's incomplete adjuvant, and 14 days after the last injection antibodies against the microbial vaccine were determined in the serum. Group 2 received an intravenous injection of $5 \cdot 10^8$ sheep's red cells and the serum level of hemagglutinins and hemolysins was determined 5 days later. Group 3, to estimate the effect of tolerance to *C. albicans* on transplantation reactions, underwent transplantation of skin allografts. Groups 4 and 5 were immunized by a single subcutaneous injection of 10^{10} *C. albicans* cells in 0.2 ml Freund's incomplete adjuvant, and 14 days later the spleen was taken from one group for investigation whereas skin allografts were trans-

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TABLE 1. Effect of Immunological Tolerance to *C. albicans* in CC57BR Mice on Allograft Rejection Reaction (M ± m)

Animals	Length of survival of allografts (in days)	P
Tolerant	47,4±4,3	<0,001
Tolerant, immunized with C. albicans	42,6±4,1	<0,001
Intact	9,4±0,41	—
Intact, immunized with C. albicans	6,8±0,25	—

TABLE 2. Number of Lymphocytes Giving Immunoadhesion Reaction of Tolerant and Intact CC57BR Mice Immunized with *C. albicans* Vaccine (per 10^6 lymphocytes; M ± m)

Animals	Rosette-forming cells with C3H mouse red cells	Lymphocytes with adherent cells
Tolerant, immunized with C. albicans	880,0±74,2 $P<0,001$	4575,0±327,0 $P<0,001$
Intact, immunized with C. albicans	7600,0±620,0	31458,0±2420,0
Intact	187,0±12,6	490,0±38,6

planted on to the other group. Each experimental and control group consisted of 25 mice.

Eight samples of serum (each sample being a mixture of serum from three mice) and of suspensions of spleen cells obtained individually from eight mice of each experimental and control group were tested.

Antibodies were determined in the serum by the agglutination test with cells of *C. albicans* vaccine and by hemolysis and hemagglutination tests with sheep's red cells.

The suspension of spleen cells was prepared in the usual way [2]. The number of lymphocytes reacting with the donor's antigens and antigens of the *C. albicans* vaccine was determined: 1) by the number of rosettes with donor's red cells [5], 2) by the number of lymphocytes to the surface of which at least four cells of *C. albicans* vaccine adhered [7], 3) by the blast-transformation reaction (BTR) of lymphocytes after cultivation of 10^7 spleen cells for 48 h with 10^6 cells of the *C. albicans* vaccine and donor's lymphocytes in medium No. 199 with 20% calf serum; the BTR was read and the intensity of incorporation of thymidine-H^3 (specific activity 10 Ci/mmole, "Izotop") into lymphoid cells measured with a Nuclear Chicago scintillation counter [3], and 4) from the level of cytotoxic activity of the lymphocytes relative to a culture of C3H fibroblasts [2].

The statistical analysis of the results was carried out by Student's method.

EXPERIMENTAL RESULTS

Injection of *C. albicans* vaccine by the above-mentioned scheme into newborn CC57BR mice completely suppressed antibody formation against that microbial antigen after four injections of vaccine into the mice, whereas the titer of antibodies in the control vaccinated mice was not less than 1:128. This pointed to the development of immunological tolerance in the mice to *C. albicans* antigen. The suppression of the immune response to *C. albicans* antigens observed in the experimental group of mice was specific, for hemolysin and hemagglutinin formation was not suppressed after immunization of these mice with sheep's red cells.

Investigation of the effect of the immunological tolerance to *C. albicans* obtained in this way on transplantation reactions showed considerable lengthening of the life span of the allografts in tolerant CC57BR mice (Table 1). Some grafts survived more than 3 months. Similar results were obtained by allografting skin on to tolerant recipients immunized 14 days previously with *C. albicans* vaccine, indicating preservation of the tolerance. Meanwhile, immunization with *C. albicans* vaccine caused acceleration of graft rejection in nontolerant recipients (Table 1).

Inhibition of transplantation reactions in mice tolerant to *C. albicans* was specific, for it was absent after corresponding treatment of newborn mice with *Escherichia coli* vaccine not containing cross-reacting antigens with the donor's tissues.

The number of rosette-forming cells and lymphocytes in the spleen of tolerant mice immunized with *C. albicans* vaccine, capable of attaching microbial cells to their surface, was 10 times smaller than in vaccinated mice of the control group (Table 2).

TABLE 3. Cytotoxic Activity of Lymphocytes of Tolerant and Intact CC57BR Mice Immunized with *C. albicans* Vaccine (M ± m)

Animals	Number of living fibroblasts ($\cdot 10^2$)	Cytotoxic activity (in %)
Tolerant, immunized with C. albicans	1216,7±96,3 $P<0,001$	37,4
Intact, immunized with C. albicans	200,8±18,2	90,4
Intact	2090,0±106,0	—

Comparison of the activity of the lymphocytes of the tolerant and nontolerant mice immunized with *C. albicans* in the BTR showed that the activity of the lymphocytes in the nontolerant mice was 2-3 times higher than in the tolerant animals. The intensity of thymidine-H^3 incorporation into lymphocytes after incubation of lymphocytes of immunized nontolerant animals with *C. albicans* antigen was 107.0 ± 9.5 counts/min, but if immunized with donor's lymphocytes it was 148.3 ± 9.6 counts/min, compared with 30.9 ± 3.0 counts/min in the control ($P < 0.001$). Activity of the lymphocytes in the BTR in vaccinated tolerant mice after incubation with microbial or tissue antigens did not exceed 47.0 ± 4.1 counts/min (34.1 ± 3.0 counts/min in the control; $P > 0.1$).

As Table 3 shows, cytotoxic activity of the lymphocytes against allogeneic target cells in tolerant mice immunized with *C. albicans* vaccine was 2-3 times less than in the control group of immunized animals.

Immunological tolerance to transplantation antigens induced by *C. albicans* vaccine in newborn mice was transferred to 15 CC57BR mice irradiated in a dose of 700 R by intraperitoneal injection of $2 \cdot 10^8$ spleen cells of tolerant animals. In the control series 30 irradiated recipients received syngeneic spleen cells of normal mice. On the day after transfer, the experimental and some of the control animals were immunized with *C. albicans* vaccine, and 14 days later all the mice were grafted with skin from C3H mice. In recipients receiving lymphocytes from tolerant donors, the life span of the allografts was increased after immunization (to 22.4 ± 4.1 days), whereas in the control group rejection was accelerated (to 6.3 ± 0.4 days; 9.6 ± 0.51 days in the control group of unvaccinated mice; $P < 0.001$).

These results indicate that treatment of newborn mice with *C. albicans* vaccine containing cross-reacting antigens is accompanied by the development of immunological tolerance to the microbial antigen and to the evidently related donor's transplantation antigen and leads to a marked increase in the survival period of skin allografts. In tolerant animals after sensitization the number of lymphocytes participating both in the reception and recognition of the antigen and also directly in the destruction of allogeneic tissue is reduced.

LITERATURE CITED

1. R. P. Ogurtsov, Abstracts of Proceedings of the Fourth All-Union Conference on Immunopathology [in Russian], Leningrad (1973), pp. 74-76.
2. G Ya. Svet-Moldavskii, I. Yu. Chernyakhovskaya, and Z. G. Kadagidze, in: Current Problems in Immunology and Immunopathology [in Russian], Leningrad (1970), pp. 167-176.
3. B. B. Fuks and I. V. Konstantinova, Cytochemistry of Immunogenesis under Ordinary and Extremal Conditions [in Russian], Moscow (1973).
4. E. N. Fox and R. D. A. Paterson, J. Immunol., 105, 1031 (1970).
5. H. S. Micklen and N. A. Staines, Advances Exp. Med. Biol., 5, 333 (1969).
6. F. T. Rapaport, R. M. Chase, Jr., and A. C. Solowey, Ann. New York Acad. Sci., 129, 250 (1966).
7. E. Reiss, E. Mertens, and W. E. Ehrich, Proc. Soc. Exp. Biol. (New York), 74, 732 (1950).