EXPERIMENTAL USE OF ANTILYMPHOCYTIC SERUM AND MICROBIAL ANTIGENS CROSS-REACTING WITH MAMMALIAN TISSUES TO PRODUCE AND ABOLISH TOLERANCE TO TRANSPLANTATION ANTIGENS

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The combined administration of antilymphocytic serum and candida vaccine, containing common antigens with the donors' (C3H mice) tissues, into CC57BR mice inhibited the formation of antibodies against microbial antigens by related tissues and increased the life span of skin allografts. The injection of candida vaccine into CC57BR mice tolerant to alloantigens of C3H mice led to the earlier rejection of a skin graft transplanted to them from C3H mice.

Previous experiments showed that cells of Candida albicans possess antigens that are common with antigens of the mammalian skin, heart, and kidneys [1]. The ability of microbial antigens related to tissue antigens to induce immunological states and also to cause the earlier rejection of allografts has also been described [3, 6].

The object of this investigation was to study the possibility of using microbial antigens related to tissue antigens in order to produce tolerance to transplantation antigens and also to study the ability of these microbial antigens to abolish artificially created tolerance to allografts.

EXPERIMENTAL METHOD

Experiments were carried out on CC57BR (recipients) and C3H (donors) mice weighing 16-18 g. A heat-killed vaccine of C. albicans was used as the microbial antigen for immunization. The vaccine was injected subcutaneously in a dose of 10^8 bacterial cells three times at intervals of 24 h. In some experiments the microbial vaccine was injected subcutaneously in a single dose of 10^{10} bacterial cells mixed with 0.3 ml of Freund's incomplete adjuvant. Spleen cells from C3H mice were used as carriers of transplantation antigens; they were injected intraperitoneally into the recipients in a dose of $3.5 \cdot 10^8$–$4 \cdot 10^8$ per mouse. Skin allografting [4] was carried out at various times after treatment of the mice with the antilymphocytic sera (ALS) and vaccine. The index of transplantation immunity was the duration of survival of the graft, assessed macroscopically by the usual method [4]. Antibodies against the microbial antigen were estimated by the agglutination test with vaccine containing 10^9 bacterial cells in 1 ml. Antibodies against the tissue antigens were estimated by the hemagglutination and lymphagglutination tests with donor's red blood cells and lymphocytes.

The ALS was obtained by intensively immunizing rabbits with lymphocytes from noninbred mice [2]. The immunosuppressive activity of the sera was tested in vivo by their ability to inhibit hemagglutinin formation after a single subcutaneous injection had been given in a dose of 0.5 ml to mice 3 days before immunization with sheep's red cells. Sera inhibiting hemagglutinin production down to 10% of the control

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TABLE 1. Creating of Immunologic Tolerance to Skin Grafts in Response to the Combined Action of ALS and C. albicans Vaccine

Group	Agents administered at times indicated below (days)				Length of survival of graft (in days)	P
	−3	0	+30	+60		
1	ALS	C. albicans			14,8±0,2	<0,001
2	NS	» »			8,4±0,17	
3	ALS	» »	C. albicans		14,0±0,3	<0,001
4	NS	» »	» »		8,5±0,15	
5	—	» »	ALS	C. albicans	8,2±0,16	>0,1
6	—	» »	NS	C. albicans	8,0±0,14	
7	—	—	—	—	8,8±0,31	

Note. In the experimental groups transplantation was carried out 30 days after the last injection of vaccine.

TABLE 2. Effect of C. albicans Vaccine on Immunologic Tolerance to Transplantation Antigens

Group	Agents administered at times indicated below (days)			Length of survival of graft (in days)	P
	−3	0	+30		
1	ALS	C3H lymphocytes	—	18,0±0,3	<0 001
2	NS	Same	—	6,0±0,1	
3	ALS	—	C3H lymphocytes	6,3±0,1	<0,02
4	NS	—	Same	5,8±0,2	
5	ALS	C3H lymphocytes	C. albicans	8,3±0,1	>0,1
6	NS	Same	» »	8,15±0,1	
7	ALS	» »	C. albicans with incomplete adjuvant	8,7±0,1	>0,1
8	NS	» »	Same	8,5±0,2	

Note. Transplantation carried out 30 days after immunization.

level were chosen for the experiments. Before injection, the serum was exhausted with mouse red cells. Normal rabbit serum (NS) was injected into the mice of the control groups. Each experimental and each control group consisted of 20 mice.

EXPERIMENTAL RESULTS

The effect of the ALS on the survival of a transplanted allograft in CC57BR mice immunized with the microbial vaccine was determined in the tests of series I. ALS was injected 3 days before immunization. After 1 month allogeneic skin grafts were transplanted from C3H mice to some of the immunized mice. Another group of the mice were reimmunized with the microbial vaccine and transplanted another month later with skin from C3H mice. The mice of the control group received an injection of ALS 1 month before immunization with the microbial vaccine or 1 month before grafting without preliminary sensitization with the microbial antigen. The injection of ALS 1 month before immunization with the microbial vaccine did not affect antibody production induced by it. Injection of ALS 3 days before the first cycle of immunization completely suppressed antibody formation against the candida antigen. When these mice were reimmunized with the microbial vaccine the antibody titer was considerably lower than in the control groups of animals reimmunized without the preliminary injection of ALS (1 : 4 and 1 : 72, respectively). Only the sera of the control groups of mice reimmunized with the candida vaccine reacted with the tissue antigens (maximal titer 1 : 32). No antitissue antibodies were found in the mice treated with ALS, even after revaccination.

Injection of candida vaccine 3 days after administration of ALS (Table 1) prolonged the survival of the allografts (Group 1). A slower rate of graft rejection was found in these animals also after reimmunization with candida vaccine (Group 3). The injection of ALS into unimmunized mice 1 month before grafting did not affect the rate of rejection of the grafts. There was likewise no increase in the period of survival of the graft if the ALS was injected 1 month after immunization and, correspondingly 1 month before revaccination with the microbial vaccine (Group 5).

It can be concluded from these results that ALS inhibits the immunologic response to microbial and tissue cross-reacting antigens. The combined administration of ALS and C. albicans antigen reduces the ability of the animal to respond immunologically to the related transplantation antigens of the donor. In that case ALS was effective only if given before the primary immunization. The inhibitory action was not connected with the direct effect of ALS on transplantation, for the skin grafting was carried out 30-60 days after injection of the serum before the latter had had time to act.

In the experiments of series II an attempt was made to abolish the artifically created tolerance to the donor's alloantigens. Immunologic tolerance was obtained by injecting ALS into CC57BR mice 3 days before they were injected with lymphocytes from C3H mice by Monaco's method [5] and this led to a marked increase in the length of survival of allografts from C3H mice transplanted 1 month later (Table 2, Group 1). The graft survived about 70 days on some of the animals. Tolerance did not arise if the ALS was injected 1 month before injection of C3H lymphocytes.

To abolish the tolerance created in the CC57BR mice they were immunized (1 month after receiving an injection of donor's lymphocytes) with C. albicans vaccine. The animals were grafted with allogeneic skin 2 weeks after immunization. Immunization with C. albicans vaccine in physiological saline or in incomplete Freund's adjuvant interrupted the state of tolerance to the transplantation antigens and this was reflected as shortening of the life of the allografts (Table 2, Groups 5 and 7).

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