# USE OF GERM-FREE MICE IN EXPERIMENTAL TRANSPLANTOLOGY

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The need to use immunodepressants in transplantation of allogeneic organs makes it essential to conduct research into conditions for management of recipients under which they will run the least risk of developing infectious complications. Besides worsening of the patient's general condition due to the harmful action of pathogenic factors on the recipients, the graft rejection process may be intensified on account of infectious and helminthic agents, due to the appearance of T cells and cytotoxic antibodies, directed toward determinant antigens common for the microorganism and the host [1, 2, 5, 6]. One way of settling this problem is by prophylactic disinfection of the recipients by the use of antibacterial, antiviral, and antihelminthic agents [3]. Problems relating to the experimental production of germ-free animals have been discussed elsewhere [4, 10, 11].

The aim of this investigation was to compare survival of a skin allograft in germ-free mice, kept under conditions of isolation, and in mice bred in the usual way and kept in the animal house.

## EXPERIMENTAL METHOD

Mice weighing 21 g of the following lines were used: A/Ola (36 mice), BALB (70 mice), and C57BL/6 (20 mice). The recipients were A/Ola (Haplotype H-$2^a$) and BALB (haplotype H-$2^d$) mice, and the skin graft donors were C57BL/6 mice (haplotype H-$2^c$). The A/Ola mice and some of the C56BL/6 mice were germ-free (bred at the Svetlye Gory nursery) and kept under sterile conditions [4]. Transplantation was carried out under general anesthesia (pentobarbital 0.2 ml, in a concentration of 50 mg/ml, intramuscularly). The operations on the germ-free mice were performed in a sterile bench-top container. The animals' sex was taken into account for skin grafting. Pieces of skin from the donor's tail, measuring 6 × 8 mm, were grafted in the region of the recipient's spine. A no-suture method of transplantation was used and the skin graft was fixed with a gauze dressing and a wide strip (3 cm) of adhesive tape, so that the edges of the tape overlapped each other by 2-2.5 cm. The adhesive tape was removed on the 6th-7th day after transplantation. The state of the graft was assessed by visual observation. Immediately after transplantation some of the animals were given an intraperitoneal injection of 200 mg/kg of cyclophosphamide (CP). Further injections of CP were given once a week in a diminishing dose (100, 50, and 25 mg/kg). The results were subjected to statistical analysis.

The animals were divided into six groups. BALB mice (n = 29) were kept under ordinary animal house conditions and were grafted with skin from C57BL/6 mice without subsequent injection of CF (group 1). Other BALB mice (n = 41) received an allograft, as well as injections of CP (group 2). Germ-free mice of the A/Ola line (n = 13), kept under sterile conditions, received an allograft from germ-free C57BL/6 mice, but no injections of the immunodepressant (group 3). Germ-free A/Ola mice (n = 9) received a skin graft from germ-free C57BL/6 mice and also injections of CP (group 4). Germ-free A/Ola mice (n = 5), kept under ordinary conditions pre- and postoperatively, received a skin graft from C57BL/6 mice, kept under animal house conditions and receiving injections of CP (group 5). A/Ola mice reared in the normal way (n = 9) received a skin graft from C57BL/6 mice, also reared in the ordinary way, without any CP injections (group 6).

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TABLE 1. Length of Survival of Skin Allografts on Mice of Different Groups

| Group of animals | Line of mice and experimental conditions | Survival of Allograft, days |
|---|---|---|
| 1 | BALB/c without CP | 14,38±0,3 |
| 2 | BALB/c with CP | 21,15±0,37 |
| 3 | A/Ola from sterile conditions, without CP | 17,92±0,58 |
| 4 | A/Ola from sterile conditions, with CP | 26,78±1,95 |
| 5 | A/Ola from sterile conditions, kept in ordinary conditions before and after operation, with CP | 29±3,5 |
| 6 | from ordinary conditions, without CP | 18,77±0,74 |

## EXPERIMENTAL RESULTS

In BALB mice of group 1, which constituted the main control, the allograft was rejected 12-18 days after the operation. In mice of group 2 (also BALB), receiving a skin graft and CP injections, rejection took place between the 12th and 29th days. The graft survived longest (27-29 days) on five of the 41 animals (Table 1). In the germ-free A/Ola mice of group 3, kept under sterile conditions, skin grafted without receiving the immunodepressant, the grafts were rejected between the 16th and 21st days. In germ-free mice of the same line in group 4, which received CP, the graft was rejected later (between the 19th and 36th days). In germ-free mice of group 5, kept pre- and postoperatively under ordinary conditions, the length of survival of the allograft also exceeded that for BALB mice reared in the ordinary way (22-39 days compared with 12-29 days). In group 6, consisting of A/Ola mice reared in the ordinary way, the graft survived 16-21 days.

The following conclusion can be drawn by comparing the length of survival of the skin allograft on animals of different groups. The skin graft on animals of different lines (A/Ola and BALB) reared in the normal way, in experiments without CP injections, was injected early (after 16-21 and 12-18 days respectively). CP injections given to BALB mice reared in the normal way lengthened the survival of the graft to 12-29 days. Injections of CP into germ-free A/Ola mice lengthened the period of survival of the graft to 22-39 days (compared with 16-21 days without CP injections). Germ-free mice, transferred to ordinary conditions, evidently preserve their germ-free status for some time, and for that reason the length of survival of the allograft on these animals, treated with CP, also continued to exceed that on ordinary BALB animals (22-39 days compared with 12-29 days).

In cases when germ-free mice kept in sterile containers were used, none of the animals died from infectious complications if the immunodepressant was given, whereas mice kept under animal house conditions frequently died with characteristic manifestations of infection (conjuctivitis, diarrhea, skin lesions).

The use of germ-free animals in allografting experiments thus enabled the length of survival of both animals and graft to be increased. The mechanism of graft preservation has not been finally explained and requires independent study. The decisive role in this situation may perhaps be played by absence of sensitization to cross-reacting antigens common to the tissues of the host and pathogenic microorganisms [7, 8].

Despite difficulties in the organization of condictions for keeping germ-free animals, their advantages are evident, especially when long-term experiments involving administration of immunodepressants are to be undertaken.

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