HOMOTRANSPLANTATION OF SKIN IN RATS AFTER TREATMENT OF DONORS WITH SPLEEN SUSPENSIONS FROM RECIPIENTS

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Despite considerable success in the study of transplantation immunity, the problem of tissue incompatability is far from resolution. Although it is possible experimentally to achieve acceptance of incompatible transplants by using means to inhibit the defensive immunologic reaction of the macroorganism, their use clinically is limited for the same reason.

In this connection, the overcoming of tissue incompatibility by altering the state of the transplanted tissue deserves detailed investigation. Influence on the isolated transplant, outside the exchange of substances with the surrounding medium, appears poorly effective at the present. Until now the cultivation of tissues in vitro has been unsuccessful and in addition, cultivation of organs with directed changes in the antigenic structure of the cells. The problem of the possible influence on the future transplantant via the donor organism appears of special interest.

In earlier work we found that when tumor-bearing rats or mice were injected with suspensions of normal tissue from animals of the recipient species, the tumor possessed the capacity for growth under conditions of heterotransplantation [4]. Thus the regulation of tumor transplantation and normal tissue transplantation are similar in many ways and we resolved to try a similar method for homotransplantation of normal tissue.

In this work we studied the influence of preliminary treatment of donors with splenic suspensions from the recipients on acceptance of skin in rats.

METHODS

The experiments were performed on adult, non-pedigreed rats of five to six months in age that weighed 200-300 g and were not closely related. The transplantation of skin was performed without regard for the sex of donor and recipient. Under ether anesthesia and aseptic conditions, half (or slightly more) of the spleen was removed from the future recipient. Fragments were cut with scissors and ground in a mortar and a five-fold volume of physiological solution was a added to the pulp obtained. Immediately after preparation of the suspension, 1.5 to 2 ml was injected into the future donor subcutaneously and intraperitoneally. Subsequently two to three injections were made every two to four days (suspension was kept at 3°). At two to eight days after cessation of the injections, a piece of abdominal skin (from 1.5 to 6 cm^2) from the thusly prepared donor was removed under aseptic conditions, carefully freed from fascia and cellular tissue and implanted on a prepared side (of somewhat larger size) on the back of the recipient. Since some pieces were damaged by the animals and sloughed in the first days, to judge "true" rejection occurring as the consequence of immune reaction by the recipient, we usually made two or three transplants on each rat (a total of three to eight cm^2 of skin). Transplants of larger size (up to six cm^2) were attached with a continuous suture and covered with a gauze tampon which was attached to the skin with silk. Transplants of smaller size (up to two cm^2) were laid on the site and their edges glued to the skin of the recipient (with "Ago" glue).

Skin was transplanted to 23 rats after preliminary injection of donors with spleen suspensions from recipients and to three rats after two to three transplants of future recipient skin to the donor.

RESULTS

In eight rats the fragments of skin perished in the first days (in three of suppuration, in five—from technical errors). In 18 rats the transplants survived, however, in three they became necrotic on the 10th, 16th and 17th day, (two of these after transplantation of recipient skin to donor). In two other rats the transplants were in good condition (they bore hair) until the 23rd and 26th day, after which small ulcerations appeared in their centers and rapidly enlarged, and in three to four days the entire surface of the transplants had ulcerated. In the remaining 13 rats the fate of the surviving transplants was the same. After they took, root hair gradually grew out, but more sparsely than on the surrounding skin of the recipient. This, evidently, is explained by the fact that the transplants were taken from the belly of the donor where the hair is more delicate and sparse than on the back of the recipient. The transplants were elastic and mobile. In certain cases their surface was peeling; sometimes on the 25-40th day small ulcers appeared which rapidly healed.

Areas occupied by transplants did not remain stable. Usually after one and one-half to three months post transplantation a considerable diminution in their size was observed, which at four to five months from transplantation of a small size (up to four cm^2) only shriveled strips of skin remained covered with scant hair which later did not change markedly (after 6-8 months) (Fig. 1, a and b). In other instances the transplanted fragments almost completely preserved their initial size.

Transplants of larger sizes also gradually diminished (two to three times) but their entire borders were well seen at five to seven and even at ten months (note Fig. 1, c). (Further observation was not always possible because of the natural death of the animals).

Study of sections of the transplants at different periods after transplantation permitted us to confirm the fact of their true acceptance. Even after three to six months differences in the structure of transplant and recipient skin were preserved. The collagen fibers of the dermis in the recipient were coarse, and lay in various directions, forming "whorls" whereas in the transplant taken from the donor belly they were delicate, fine and arranged in single plane. The hair bulbs in the transplant were encountered comparatively more rarely than in the recipient skin; in the connective tissue of the graft many fibroblasts were found. Cell "cuffs" were often found surrounding vessels and consisted of histiocytes, plasma cells and lymphocytes, which, evidently, indicates the continuing reorganization of the transplant. These cells probably may synthesize antibody to the transplant antigen (Fig. 2).

On the backs of three females were grafted fragments of skin from the mammary glands. In three to four months, upon microscopic study, we succeeded in finding portions of preserved milk glands (Fig. 3).

We also undertook to obtain the cross-grafting of skin from white non-pedigreed rats and August strain rats after injection of recipient spleen suspensions into the donor. However, in carrying out six grafts we obtained negative results: the skin sloughed in 12-14 days, which may be explained by the imperfections in the method of preparing the donors.

Simultaneously with experiments in which the donors were given spleen suspensions from the future recipients we carried out skin grafts on control rats which did not undergo preparation before transplantation. Fifteen pairs of white, non-pedigreed rats underwent operation. Usually the transplants were mummified and sloughed by the 10-15th day. These data agree with the results of experiments by several authors [2-3] which showed that in skin homotransplantation in adult (three to four-month) white, non-pedigreed rats, which were not closely related, transplants of moderate size (up to six cm^2) died on the ninth to 14th day. At the same time larger homotransplants (30-60 cm^2) may live considerably longer [2] as a consequence of partial suppression of the immune reaction by the excess of antigen and by intense operative trauma. On the other hand, the survival period of small transplants also may be greater than that of medium sized transplants, since the antigenic stimulus from their side may be weakened. Such lengthening of the life of transplants (up to 18 days) we have observed in two control rats which were grafted with small pieces of skin (up to 1 cm^2).

Thus, after preliminary injection of donor-rats with spleen suspensions from prospective recipients, a true acceptance of skin homotransplants was observed in the majority of cases with subsequent reorganization and in particular, replacement by the tissue of the recipient. It is improbable that with this, considerable reorganization of the antigenic structure of the donor tissue took place, bringing about the overcoming of incompatibility. However, some components of the tissue from the prospective recipient injected into the donor with the suspension, may have been absorbed on the latter's cells and then evoked quantitative or qualitative changes in the isoantigenic structure of the

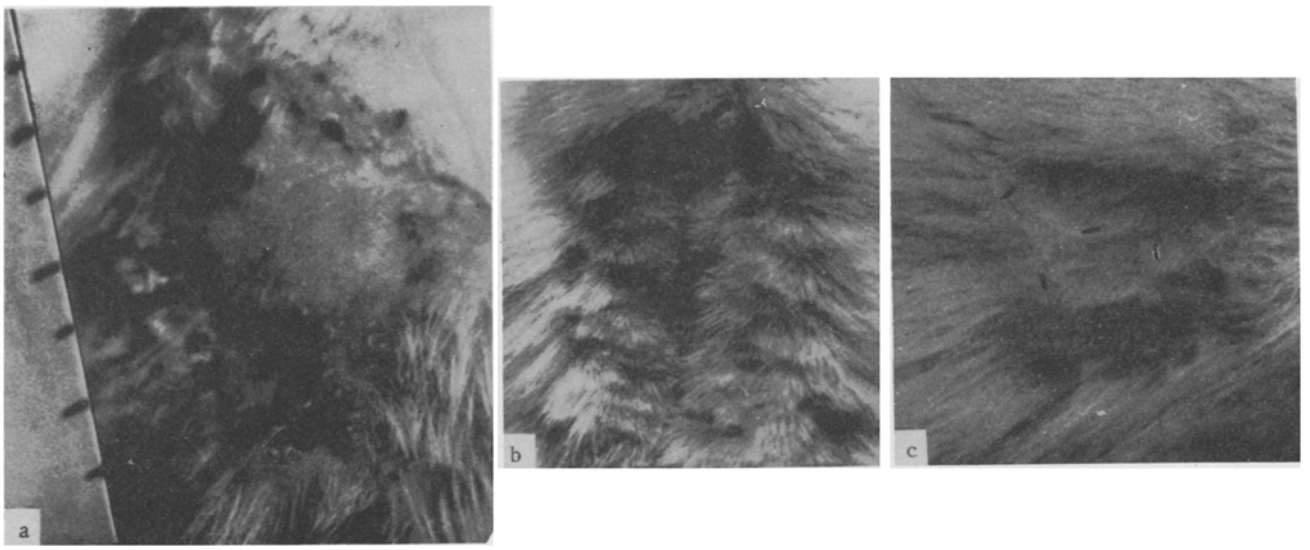

Fig. 1. Homotransplant of skin in rat at different postoperative times. Black marks at the edge—surgical sutures. a) Transplant in rat No. 53 at 25 days after transplantation; b) same, at 3 months; c) transplant in rat No. 6 at 10 months after transplantation.

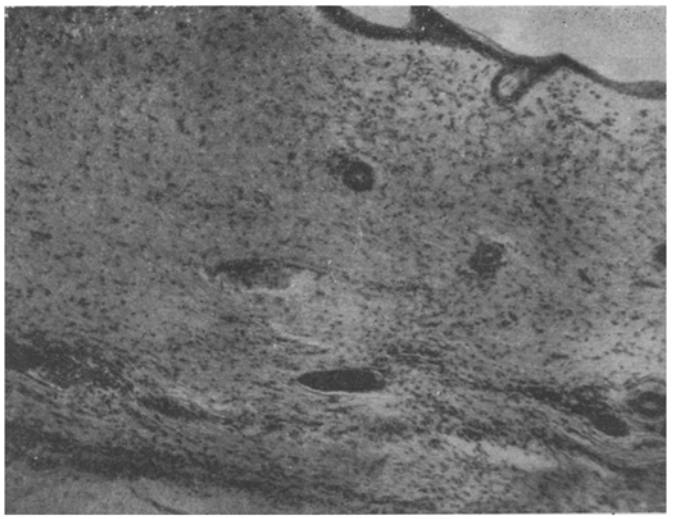

Fig. 2. Transplant in rat No. 36 at four months after transplantation. Cellular perivascular "cuff." Hematoxylin-eosin stain. Ocular × 7, objective, × 8.

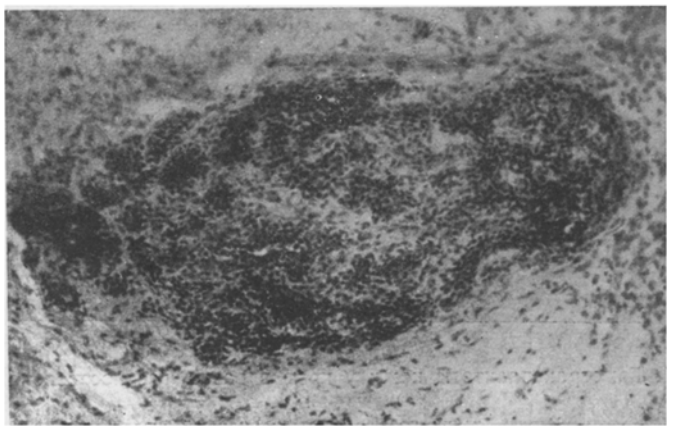

Fig. 3. Transplant in rat No. 59 at $3\frac{1}{2}$ months. Fragment of preserved mammary gland of the donor. Hematoxylin-eosin stain. Ocular × 7, objective, × 10.

cells in the prospective transplant. This, possibly, is the reason for the decreased immunogenic activity of the transplant which conditioned its acceptance.

In this connection it must be noted that many authors [1, 5-7] have observed a prolonged life-span for incompatible transplants after their treatment with serum from the prospective recipients. It is entirely probable that the use of nuclear components of cells(components which carry genetic informations, for example, nucleoproteins) in similar experiments will allow not only diminution of individual antigenicity of the transplanted tissue, but also procurement of directed alterations of antigenic structure which will be more compatible with the tissues of the recipient.

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