STUDY OF THE STATE OF FROZEN GRAFTS AND THE REACTION OF THE REGIONAL LYMPH GLANDS AFTER AUTO- AND HOMOTRANSPLANTATION

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Studies of the reaction of the regional lymph glands to homotransplantation of skin [3,7] have shown that an immunologic response develops regularly in them before the graft is rejected. In the spleen and lymph glands special immunologically active cells responsible for humoral and transplantation immunity are found [5]. It has been shown that after homotransplantation of skin, an increase in the number of young pyroninophilic cells of the lymphoid series – large lymphoid cells – is found in the regional lymph glands [8]. These cells are considered to be the morphological equivalent of immunoblasts – the cells responsible for antibody formation, while the smaller lymphocytes and plasma cells are the morphological equivalent of the immunocytes – the cells responsible for rejection of the graft and the production of humoral antibodies [4, 6]. Some investigators have therefore naturally attempted to use the reaction of the lymph glands as a biological test for assessing the antigenic properties of the graft [1, 2, 9].

In the present investigation, the reaction of the regional lymph glands to transplantation of autologous and homologous tissue previously frozen in various conditions was studied morphologically.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rats. Skin grafts were taken from the lateral surfaces of the chest and separated from the panniculus carnosus by means of a scalpel. The grafts were kept in 15% glycerol or 10% dimethylsulfoxide (DMSO) before freezing. The skin was then frozen in dry ice to – 70° or in liquid nitrogen to – 196°.

The variants of auto- and homotransplantation studied in these experiments are shown in the scheme (Fig. 1).

Material from the grafts was taken for histological investigation on the 5th, 7th, 10th, 14th, 18th, and 21st days after transplantation. The regional (axillary) lymph glands, from which histological sections and impressions were made, were investigated at the same times.

EXPERIMENTAL RESULTS

All the autografts (fresh and frozen) took successfully. The homografts were all identical in appearance until the 7th day and were hardly distinguishable from the autografts.

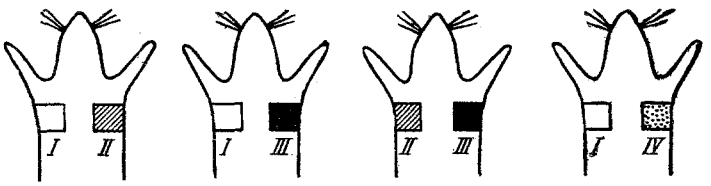

Fig. 1. Scheme of auto- and homotransplantation. I) Fresh graft; II-IV) frozen graft with 15% glycerol at – 70° and – 196° and with 10% dimethylsulfoxide at – 70°.

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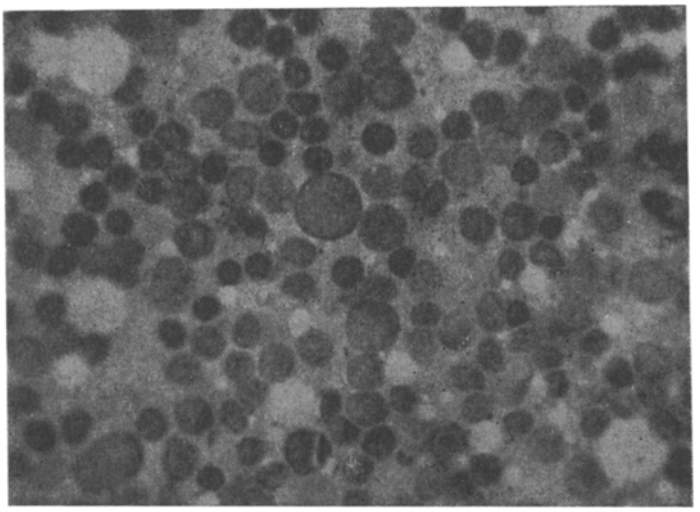

Fig. 2. Regional lymph gland on the 14th day after homotransplantation of fresh skin. Impression preparation. Azure-eosin. Objective 90x, ocular 7x.

The results of the histological investigation of the homografts at these times, however, revealed a distinct difference in the reaction of the recipient's tissues to the auto- and homografts. In the case of both the fresh and the frozen homografts, a much more violent cellular reaction to the graft was found in the surrounding tissues, and in addition, besides polymorphonuclear leukocytes, collections of lymphocytes and plasma cells were seen, mainly concentrated around the blood vessels. In the homograft itself (unlike the autograft) degeneration and death of the epithelial cells and cellular infiltration of the deep layers of the dermis could be seen. The degenerative and necrobiotic changes were more marked in the fresh homograft than in the frozen.

On the 9th-10th day the grafts were shrunken and dried. Histological investigation at these times also revealed a clear difference between the reaction of the tissues to the fresh and the frozen grafts, while in some experiments of this investigation, the beginning of detachment of the fresh homografts and their substitution by proliferating granulation tissue could be observed.

By the 12th-14th day the fresh homografts had become detached. Detachment of the frozen homografts began rather later – after the 14th-18th days. Histological examination of the homografts and surrounding tissues (if the graft was still in situ) showed obvious destructive changes in the deep layers of the dermis, accompanied by disturbance of vascularization and by cellular infiltration. The lymphoid and plasma cells were much more predominant and the circulatory disorders (stasis, hemorrhage, panvasculitis, thrombosis) were more marked in the fresh homografts than in the frozen.

Histological examinations were made of the regional lymph glands parallel with those of the grafts and at the same times.

On the 5th day after homotransplantation enlargement of the lymph glands was observed in the experiments with both the frozen and the fresh homografts. Diffuse hyperplasia was detected histologically. On the 7th day, large basophilic cells appeared in the histological sections of the lymph glands in both variants of homotransplantation. By the 9th-10th days clusters of these cells could be seen in the cortical sinuses of the regional lymph glands. Finally, by the 14th day, there was a clear difference in the number of large lymphoid cells in response to homotransplantation of fresh and frozen skin: the specific cellular reaction in the regional lymph glands in all the experiments was more marked by the end of the 2nd week after homotransplantation of fresh skin than of frozen.

By the 21st day a process of regression could be seen in the regional lymph glands in both variants of homotransplantation: sclerosis, atrophy of lymphoid tissue, degeneration of the hyperbasophilic lymphoid cells, and karyorrhexis in the reticulum cells.

The reaction in the regional lymph glands was studied also by counting the number of large lymphoid hyperbasophilic cells in impressions from the lymph glands.

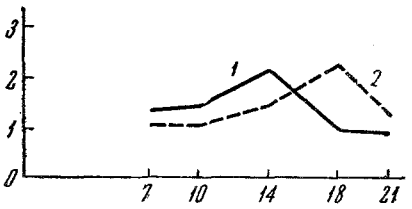

Fig. 3. Number of hyperbasophilic lymphoid cells at various times after homotransplantation of the skin. 1) Fresh graft; 2) frozen graft. Along the axis of ordinates – number of cells (in %); along the axis of abscissas – time after transplantation (in days).

The results of these investigations showed that the specific cellular reaction in the regional lymph glands was more marked on the 10th-14th day after transplantation of fresh than of frozen homologous skin (Fig. 2). On the subsequent days, conversely, the number of large lymphoid cells increased in response to homotransplantation of frozen skin and reached its maximum by the 18th day. In the later periods (21st day) this difference in the number of hyperbasophilic lymphoid cells in response to homotransplantation of the fresh and frozen skin disappeared (Fig. 3).

Besides the frozen and fresh homografts, grafts frozen to different temperatures (– 70° and – 196°), and with different protective agents (DMSO and glycerol) were also compared. No difference was found in the state of these grafts, either macroscopically or microscopically, after homotransplantation. Histological examination of the regional lymph glands likewise revealed no difference in the cellular response reaction.

On the other hand, even macroscopic observations on the autografts frozen to different temperatures and with different protective agents showed that the skin frozen to – 70° with the use of 15% glycerol took more successfully. Autografts previously treated with DMSO were drier than the surrounding healthy tissue and their color was changed.

Histological examination also confirmed that glycerol was the better protective agent: on the 21st day, the autografts were practically indistinguishable from healthy tissue, whereas in the autografts frozen in DMSO atrophic changes were observed in the epithelium lining the hair follicles.

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