RESORPTION OF HOMOGRAFTS OF TRANSITIONAL EPITHELIUM INDUCING OSTEOGENESIS IN THE SURROUNDING CONNECTIVE TISSUE

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The principles governing transplantation immunity, as determined mainly in relation to skin grafting [3, 4], are evidently of general importance in cases of the homotransplantation of epithelial tissues. Many facts have been obtained which show that when an antigenic stimulus, arising from a graft, reaches the lymphoid tissue and produces immunological changes therein, and the recipient's lymphocytes gain access to the homograft, the latter is invariably rejected. This demonstrates that when the recipient's connective tissue remains in an unchanged state around the graft, only the behavior of the lymphoid tissue is essential for the reaction of transplantation immunity. The problem, however, arises: how does the reaction of transplantation immunity proceed if the surrounding connective tissue is in an unusual state, and in particular, against the background of an induction process caused by the graft itself and giving rise to osteogenesis in the surrounding connective tissue.

We concluded from previous investigations [1, 2] that small homografts of the mucous membrane of the urinary bladder in guinea pigs, causing osteogenesis in the surrounding connective tissue, survive for a long period of time (about 1 month).

Our object was to study the course of resorption of the homograft during induction of osteogenesis in the surrounding connective tissue, and also to examine the course of the induction process in conditions of intensive transplantation immunity directed against the tissue of the inducer.

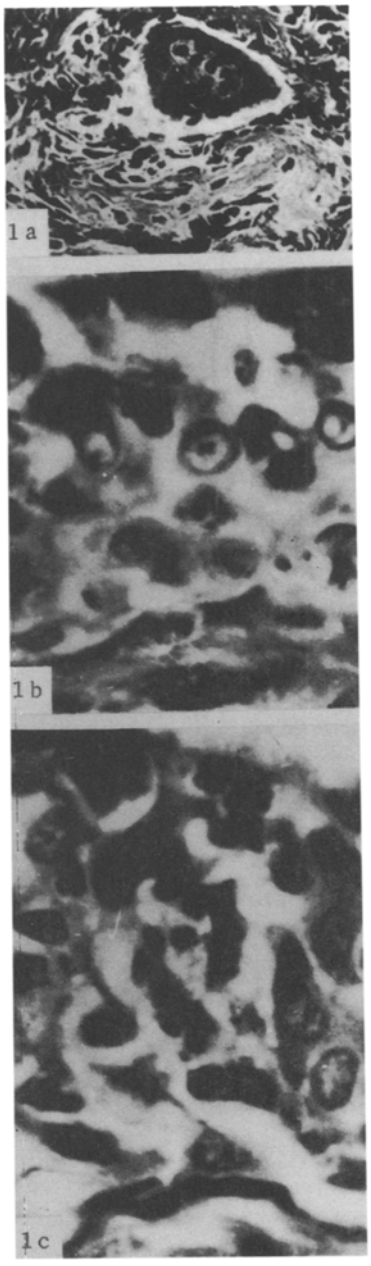
EXPERIMENTAL METHOD

Homotransplantation of pieces of mucous membrane of the urinary bladder into the anterior abdominal wall on the right side was carried out in 32 noninbred guinea pigs weighing 450 g. At the same time, three pieces of skin with a total weight of 50 mg from the same donors were transplanted into the same recipients on the left side. The animals were sacrificed on the 20th-21st day (11 guinea pigs), the 24th-27th day (14 guinea pigs), and the 29th-30th day (7 guinea pigs). The grafts of mucous membrane from the bladder with the surrounding tissue, and the skin grafts were fixed in formol and alcohol and embedded in paraffin wax or celloidin and paraffin wax. Series of sections were stained with hematoxylin-eosin and by the PAS method and counterstained with hematoxylin.

EXPERIMENTAL RESULTS

The histological picture in all the cases investigated consisted of four variants.

1. The epithelium showed no signs of degeneration and was surrounded by developing bone tissue (Fig. 1, a). The usual picture of induction was described in detail in previous communications [1, 2]. Characteristically, growth of foci of osteogenesis resulting from the continuing process of induction, i.e., conversion of connective tissue into osteogenic tissue, took place from the side of the osteogenic foci external in relation to the epithelium. In the surrounding connective tissue a very slight degree of lymphoid infiltration—both focal and diffuse—was observed, while in the osteogenic and bone tissue hardly any such infiltration was present. Lymphocytes also were found in the epithelium, but in small numbers. Mitoses were frequent among the lymphocytes in the epithelium.



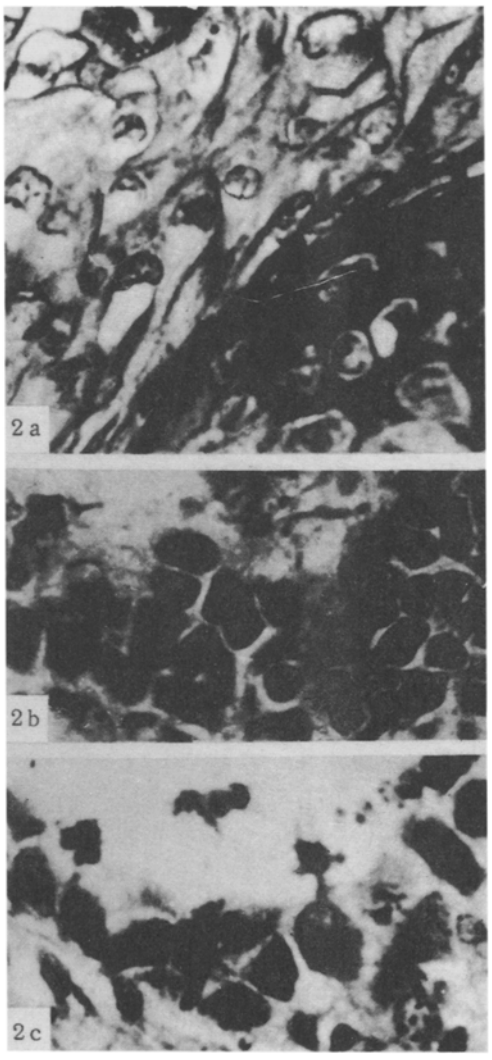


Fig. 1 Fig. 2

Fig. 1. Stages of resorption of homografts of transitional epithelium. a) On the 27th day: absence of lymphoid infiltration; b, c) on the 30th day: different phases of lymphoid infiltration and disintegration of the epithelial layer. Here and in Fig. 2: alcohol–formal, PAS–hematoxylin. Objective 24x, ocular 7x.
Fig. 2. Foci of osteogenetic induction in homografts of transitional epithelium. a) On the 27th day: epithelium and bone, free from lymphoid infiltration; b, c) on the 30th day: osteoblastic layer lining the cavity of the cyst.

2. Epithelial cysts and induced foci of osteogenesis. The epithelium and the surrounding tissue outside the area of induction were intensively infiltrated with lymphocytes (Fig. 1, b). Many of the epithelial cells and lymphocytes in these areas were in a state of degeneration. In the remaining epithelial cells of the disintegrating graft, which had lost their connection with the neighboring destroyed cells, mitoses were present (Fig. 1, c). No atypical ingrowth of epithelium deep into the underlying tissue, infiltrated with lymphocytes, took place and no fresh foci of osteogenesis were induced.

The epithelium lying above the foci of induction also contained lymphocytes, but possibly in smaller numbers than the noninducing epithelium. The osteogenic and bone tissues were practically free from lymphocytes (Fig. 2,a). In those grafts in which no induction of osteogenesis was observed, a uniform and considerable infiltration of the epithelium took place and marked degenerative changes were present in its walls. The fact was remarked upon that the smaller pieces of epithelium in the same graft were less intensively infiltrated than the walls of the neighboring large epithelial cysts.

3. Grafts in which epithelium was absent and in its place traces of lymphoid infiltration could be seen. The bone in grafts of this type was in a state of resorption and frequently only traces of it could be seen.

4. The epithelium lining the cysts completely surrounded by bone tissue was destroyed or in a state of rapid destruction and sloughing without the participation of lymphocytes. The cavity of the cysts was filled with desquamated epithelial cells and detritus, polysaccharide liberated during destruction of the epithelial cells, and also distinctive macrophages, in the cytoplasm of which products of phagocytosis of epithelial cells were found. The cavity of the cyst was lined directly by a layer of active osteoblasts, arranged in the manner of epithelium, with a strongly basophilic cytoplasm and typical "courtyard" areas between them (Fig. 2, c). This layer was followed by whole fields of active osteoblasts, among which rapid osteogenesis was taking place (Fig. 2, b). This region of a burst of fresh osteogenesis into connective tissue lying next to the destroyed epithelium could be distinguished clearly from the older bone tissue surrounding the cyst. Unlike the zone of fresh osteogenesis, the latter contained far fewer osteoblasts, although it had a well calcified ground substance and numerous osteoclasts. A very significant fact was that the front of osteogenesis in the old bone, as always, faced outward from the epithelium while the burst of osteogenesis was observed into the connective tissue facing the epithelium (of the cyst cavity). All the osteogenic tissue was free from lymphoid infiltration.

Variants 1, 2, and 3 characterized three successive stages of immunological resorption of the grafts of transitional epithelium with the participation of the lymphocytes. The distribution of these variants of the histological picture in accordance with the times elapsing after transplantation is shown in the table.

It is evident that the degree of the antigenic differences from the donor and likewise their immunological activity, varied from one recipient to another. That is why, probably, at each period grafts in different phases of resorption could be seen. Similar results have been obtained by other investigators [5], and also by ourselves in experiments in which resorption of subcutaneous skin grafts tool place (these results are described separately).

In contrast to the skin grafts, in which the degree of lymphoid infiltration and resorption of the epithelium was usually uniform throughout, the grafts of transitional epithelium showed certain special features. The subepithelial connective tissue of these grafts also was infiltrated with lymphocytes, but no infiltration was present in the induced osteogenic tissue. Furthermore, the degree of infiltration in the epithelium lying above the foci of induction was possibly slightly less than in the epithelum away from the zone of induction. Counts of the lymphoid cells in the epithelial layer, however, failed to reveal statistically significant differences in this respect. In all conditions, in the later stages of immunological resorption the differences, if present at all, were very slight and did not prevent resorption of the inducing epithelium.

Besides the ordinary process of immunological resorption with the participation of infiltrating lymphocytes, a distinctive process of rapid disintegration and desquamation of the epithelial cells of the graft was observed, so that the epithelium was protected against massive lymphoid infiltration by a continuous barrier of bone tissue.

The mechanism of this resorption is not clear. It may be associated with the action of humoral factors, and be manifested only when their concentration is high. Conditions favoring resorption may arise as a result of the fat that, because of the obstacle to lymphoid infiltration, the grafts surrounded by induced bone tissue are preserved, whereas low concentrations of humoral immunological factors, together with lymphocytes, may prove sufficient to destroy ordinary homografts. The rapid destruction of the epithelium in the conditions examined above is comparable to the resorption of secondary grafts, taking place without any significant participation of lymphocytes but in the presence of a high level of immunity.

Distribution of Grafts According to Stages Depending on Degree of Resorption of Epithelium

Variant of histological picture (degree of resorption)	Time of fixation (in days)		
	20-21	24-27	29-30
	Number of grafts		
1	4	5	1
2	3	2	1
3	1	4	4
4	3	3	1

During rapid resorption of the epithelium a large amount of osteogenetically active substances contained in the epithelium is evidently liberated, leading to a burst of osteogenesis in the connective tissue around the resorbed epithelium.

The results obtained confirm the previous conclusion [1, 2] of the dependent character of induced ectopic bone. In order to maintain existing differentiation of bone, the continuing action of the inducing factor is necessary. If the homografted tissue of the inducer is resorbed, the bone is also resorbed (i.e., is loses its specific skeletogenic differentiation), even though it is formed from the recipient's own tissue and is not subjected to the action of transplantation immunity.

SUMMARY

Experiments were made on guinea pigs. A study was made of the successive stages of resorption of transitional epithelium homografts, causing induction of osteogenesis in the recipient's surrounding connective tissue. Bone induction does not prevent resorption of the homograft. Evidently massive epithelial destruction causes the appearance of substances active with respect to induction, which provoke intense osteogenesis.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
