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DEVELOPMENT OF INTERFOLLICULAR EPIDERMIS ON SURFACE OF COLLAGEN SKELETON OF THE DERMIS: EXPERIMENTAL STUDY

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KEY WORDS: interfollicular epidermis; collagen skeleton of dermis; transplantation

Studies of healing of skin wounds in mammals [1] and experiments involving transplantation of mouse skin cells [7, 8] have revealed a phenomenon of regeneration of epidermal derivatives. A fundamental problem in this connection is the discovery of the cellular sources and conditions essential for their secondary formation, and to establish whether the keratinocytes covering the stratum papillare of the dermis can undergo transdifferentiation, with subsequent participation in the formation of derivatives.

The aim of this investigation was to study the state of keratinocytes of the interfollicular epidermis (IFE) of sexually mature rats, responsible for secondary filling of the hair follicles of the acellular collagen skeleton of the dermis (CSD) after autografting.

EXPERIMENTAL METHOD

Experiments were carried out on 34 noninbred male laboratory rats weighing 190-220 g. The transplantation technique included obtaining the CSD, preparing a receptive bed, and detachment and transplantation of the IFE.

All manipulations requiring anesthesia or immobilization of the animals (formation of the receptive bed, vacuum separation of the epidermis, wound dressing, sacrifice) were performed under ether anesthesia. To prepare the CSD a full-thickness skin graft measuring 4 × 6 cm was excised from the abdominal wall of a sacrificed rat weighing 140-170 g, fixed around the edge to a glass frame, and immersed for 24 h in a 1.5-2% solution of sodium carbonate at 36°C. The epidermis and its derivatives were then removed mechanically from one side of the graft, and remnants of the subcutaneous fatty areolar tissue from the other side. For several hours the dermal graft was washed in water and immersed for 2-24 h in medium 199 with gramicidin (20 mg/100 ml medium). This treatment of the skin graft completely removed all cells, while leaving the collagen skeleton relatively unchanged. To prepare the receptive bed, the hair was removed in the region of the anterior third of the spine. A circular skin incision is made in the interscapular region down to subcutaneous fatty areolar tissue, and 2 cm in diameter. The base of a protective chamber was sutured to the outer edge of the incision. A gauze pad was applied to the skin graft, left inside the

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Fig. 1. Filling of IFE of hair follicles of CSD by keratinocytes on 5th day after transplantation. Cells labeled with ^{3}H-thymidine visible. Hematoxylin. 200×.

chamber, and a cover fitted on the base of the chamber. The IFE was detached on the right flank of the same rat by means of a special vacuum chamber, over an area of about 2 cm^2. The liquid was removed from the vesicles thus formed. A thin layer of dermatome glue was applied from an atomizer to the stratum cornium of the detached epidermis and a semisoluble underlay was applied from above. The cover was taken off the protective chamber and the full-thickness skin graft removed together with the subcutaneous muscle. An area of CSD measuring 1 × 1.5 cm was placed on the surface of the cellular tissue of the receptive bed. The underlay was detached from the donor area (together with separated and adherent IFE) and transferred to the receptive bed, where it was held by the epidermis (basal surface) on the surface of CSD. The underlay was removed later and one layer of gauze and a dry gauze pad were placed on the epidermis. A perforated cover was fitted on the protective chamber. Once every 1-2 days the gauze pad was changed, leaving the single layer of gauze as undisturbed as possible. This layer of gauze was removed 7-9 days after transplantation. A more detailed description of the apparatus and method of its use in experimental epidermoplasty was given in [2]. For histologic investigation of the structure of the epidermal graft a strip of granulation was removed from the wound surface between 3 and 28 days after transplantation together with the CSD and epidermis, and fixed in a formalin–acetic acid mixture (after V. Ya. Brodskii). The tissue was embedded in paraffin wax. Sections 6-7 μm thick were obtained and stained with Rematoxylin and eosin. For autoradiographic detection of DNA-synthesizing cells, 1 h before sacrifice 20 animals were given an intravenous injection of 7 mBq of ^{3}H-thymidine in 1 ml isotonic sodium chloride solution. Histologic sections through the tissues of these animals, after dewaxing, were coated with photographic emulsion, exposed, developed, and stained with hematoxylin.

Fig. 2. Filling of hair follicles of CSD throughout their depth by keratinocytes of transplanted epidermis and their differentiation on 8th day after transplantation. Hematoxylin and eosin. 90×.

EXPERIMENTAL RESULTS

Several stages in the course of the regular processes taking place in the receptive bed with transplanted IFE and CSD can be distinguished.

In the early stages (3-5 days after transplantation) the epidermis, applied to the surface of CSD and retaining its viability, developed and became more stratified and differentiated. Some of its cells filled the emptying hair follicles, migrating deeply along their walls, and like the keratinocytes of the epidermal graft remaining on the surface, they commenced the mitotic cycle (Fig. 1). This led to the formation of a folliclelike structure. In turn, hematogenous cells penetrated from the surface from the receptive bed into the space between the collagen fibers.

Compared with all other times, within the time interval 6-9 days many hair follicles appeared to be most completely filled by keratinocytes, which, judging by DNA synthesis, not only continued to undergo not only mitotic division, but also differentiation in a direction typical of their natural arrangement. Keratohyaline granules were formed, and keratinization of the cells was observed (Fig. 2). The number of cells between the collagen fibrils of the skeleton of the dermis also was increased. Their composition changed toward predominance of fibroblasts and blood vessels. At the same time, ingrowing of a wedge of granulation tissue along the edges of CSD could be seen between the surface of the latter and the epidermis. As a result, folliclelike structures formed previously were drawn out of tho hair follicles.

However, between 10 and 15 days after transplantation, despite the preceding period of development, the keratinocytes of the epidermal ingrowths remaining in the duct of the hair follicles in the skeleton of the allogeneic dermis and those located nearer to the center of the latter tissues, ceased to divide and underwent destruction. This

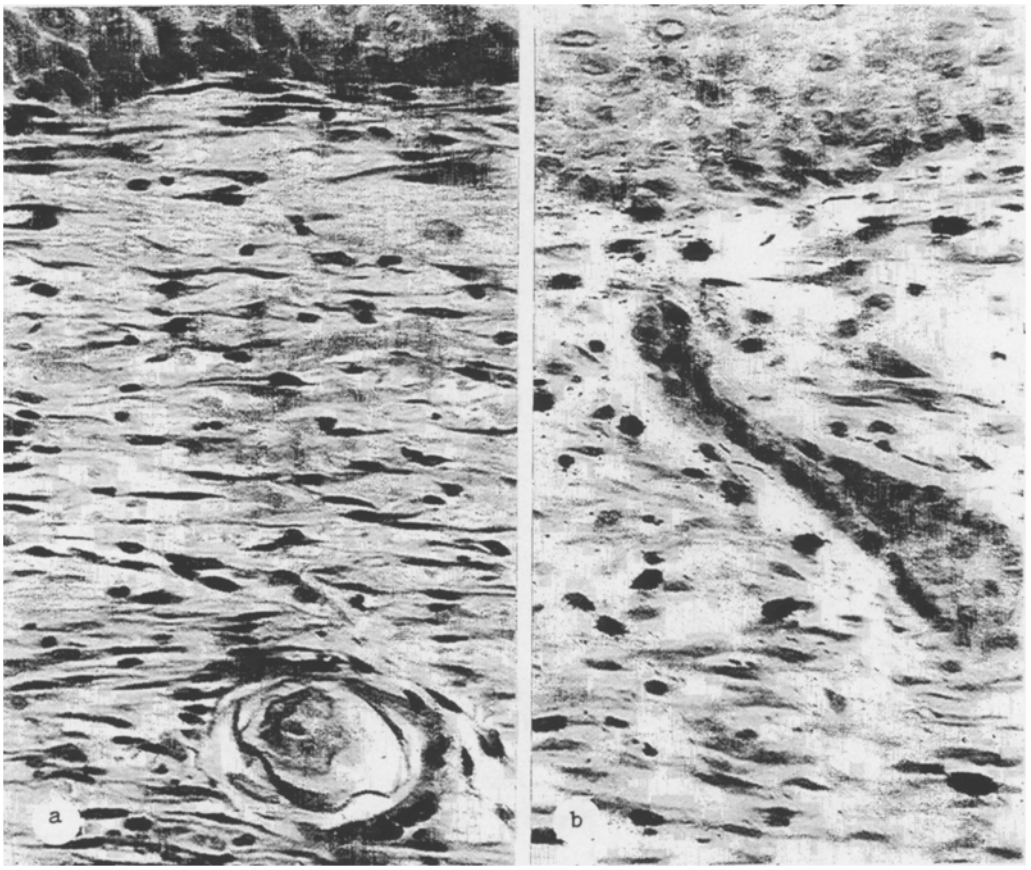

Fig. 3. Different versions of regression of ingrowths of transplanted epidermis: a) remains of keratin cells in the form of a laminated structure inside a hair follicle of CSD on 15th day after transplantation (death of epithelial cells, stained with hematoxylin and eosin, 200×); b) viable keratinocytes, some labeled with ^{3}H-thymidine, in composition of an ingrowth expelled from CSD into neighborhood of developing granulation tissue on 12th day of transplantation (hematoxylin, 200×).

was shown by a decrease in the proportion of labeled nuclei and the presence of translucent dying cells and deposits of horn cells, in the form of characteristic stratified structures (Fig. 3a). Meanwhile, some keratinocytes in ingrowths of epidermis expelled from the hair follicles in peripheral areas of CSD, and present in the neighborhood of the developing granulation tissue, not only remained viable but, judging by DNA synthesis, also continued to divide by mitosis (Fig. 3b).

Later still (starting with the 20th day) the possibility of a preceding period of filling of the hair follicles of CSD by keratinocytes of the autologous epidermis could only be guessed from the twisted contour of the basal surface of the epidermal graft. Later, even this contour was smoothed out, and the epidermal graft itself came to resemble one transplanted directly on to the surface of the subcutaneous areolar tissue [3].

Thus during filling of hair follicles of the acellular CSD by keratinocytes of IFE, no signs of transdifferentiation of the former can be seen. Regression of the follicle-like structures formed takes place in two ways. Ingrowths of epidermis, left behind in the hair follicles, undergo atrophy and destruction. Keratinocytes in the composition of the ingrowths expelled from CSD and present in the neighborhood of the developing granulation tissue evidently migrate back into the surface layer of the epidermis. This hypothesis is based on the fact that in the last case the keratinocytes not only remain viable longer, but they also continue to proliferate, although under these circumstances the length and thickness of the ingrowths formed by them are reduced.

Absence of transdifferentiation of the keratinocytes of IFE after filling of the hair follicles of CSD may perhaps be due to the sufficiently rigid determination of these cells, as a result of which their "differentiation program" cannot be changed even if the corresponding conditions are present. Another possibility is that the conditions

created were an inadequate stimulus for manifestation of all the histogenetic potentials of these cell forms. There is strong experimental evidence in support of this latter suggestion. It has been shown that connective tissue from different sources can create the conditions for development of the epidermis [5], for the repeated organization of derivatives, even from predetermined cells in perinatal mammals, specific interaction with the fibroblasts of the dermis is necessary [7, 8]. This interaction is responsible for the type of derivatives formed in embryogenesis, and for their arrangement [4]. The deeper layers of the skin are without such an influence [6].

The model suggested in this paper can be effectively used in future research aimed at studying these problems.

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EFFECT OF ANTIBODIES TO LYSOSOMAL ENZYMES ON COURSE OF EXPERIMENTAL BURN SHOCK

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KEY WORDS: burn shock; immunoglobulin fraction to lysosomal enzymes; cathepsin D; oxygen balance; acid–base state

In burn shock, marked disturbances of the microcirculation develop as a result of disorders of the systemic circulation [7, 9]. Changes in the nutritive blood flow lead to tissue hypoxia and acidosis, which lead to disturbances of the integrity of the lysosomal membrane and release of lysosomal hydrolases into the intercellular space and systemic blood flow [3, 4]. Lysosomal enzymes circulating in the blood stream during burn shock maintain and aggravate existing disturbances of the hemodynamics; in our experiments with artificially raised blood enzyme levels, injection of lysosomal enzymes into healthy animals caused inhibition of cardiac muscle activity and disorders of the microcirculation [4]. Substances inactivating lysosomal enzymes in the blood in burn shock include not only Trasylol (aprotinin) and its derivatives, but also specific antibodies to the soluble fraction of lysosomes. In experimental

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