UTILIZATION OF MONONUCLEAR CELL DNA BY THE REGENERATING EPIDERMIS

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Regeneration of the epidermis following skin autografting and allografting, and also in allergic contact dermatitis, is characterized by positive correlation between the degree of proliferation of the epidermis and the intensity of mononuclear (lymphoid-macrophagal) infiltration of its tissue [4, 5]. This correlation can be partly explained by the fact that mononuclear cells promote proliferation of regenerating cells by providing them with DNA, as many investigators accept [2, 7].

In the investigation described below this hypothesis was tested.

EXPERIMENTAL METHOD

Four series of experiments were carried out on 40 noninbred albino mice weighing 25 g. A solution of ^{3}H-thymidine with activity of 100 μCi/ml was injected intraperitoneally into 30 animals in a dose of 1 μCi/g body weight. Ten animals of this group (series I) were killed 1 h later, 10 mice (series II) remained under observation, and the remaining 10 animals (series III) were anesthetized with hexobarbital 1 h after injection of the ^{3}H-thymidine, a piece of skin measuring 1.5 × 2 cm was excised on the dorsum, and a similar piece of skin from mice of series IV, which did not receive ^{3}H-thymidine, was grafted on the fresh wound surface. In turn, skin from animals of series III was grafted on the animals of series IV. All the mice were killed 4 days later. Pieces of skin were fixed in Carnoy's fluid and embedded in paraffin wax. Sections glued to slides were coated with type M emulsion (Photographic Chemical Research Institute), exposed for 1 month at 4°C, developed, and stained with toluidine blue.

During the histological investigation the number of rows of cells, the number of mononuclear cells per 1000 epithelial cells (mononuclear index), the corresponding index for polymorphs (the leukocyte index), the number of mitotic figures per 1000 epithelial cells, the index of labeled nuclei in the epidermis (ILME; 5000 cells were counted, nuclei with at least 3 grains of silver were counted as labeled), the number of grains of silver above the nuclei of the epithelial cells, the number of labeled mononuclear cells per 1000 epidermal cells, and the number of grains of silver above the mononuclear cells were determined. The results were subjected to statistical analysis using criteria for rejection of extreme variants and Student's t-test (P = 95%).

EXPERIMENTAL RESULTS

As was expected, no differences were found in the histological structure of the skin in mice of the control series (I and II; Table 1). The malpighian layer of the epidermis of these animals consisted of two rows of cells, in which mitotic figures were extremely few and far between. Mononuclear cells were equally rare in the intercellular spaces, and polymorphs were practically absent. No significant difference likewise was found in the autoradiographic indices (both the increase in number of labeled nuclei in the epidermis of the animals of series II and the decrease in the number of grains of silver in these animals were not significant). In the epidermis grains of silver were found above the nuclei of the epithelial cells, which were distinguished most frequently by hyperchromia or were in a state of mitosis, and also above single nuclei of mononuclear cells.

Considerable changes, similar in experimental series III and IV, developed in the animals after the operations. For instance, congestion, edema, diffuse mixed cell infiltration, and degranulation of mast cells were observed in the grafted skin. Two rows of cells were still found in the malpighian layer of the epidermis, but it was infiltrated with mononuclear cells and polymorphs. The edge of the graft showed necrosis and was converted into a scab covering the wound. In the skin around the graft congestion, edema, and mixed infiltration of the dermis and hypodermis, with the formation of new capillaries and proliferation of fibroblasts, were observed. In the epidermis of the recipient the number of rows of cells in the malpighian layer increased to five, the number of mitoses was increased, and infiltration of the tissue with mononuclear cells and polymorphs was observed. The epidermis and the hypertrophied skin adnexa formed a regenerating focus, which sank into the

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TABLE 1. Changes in Epidermis of Mice depending on Time after Injection of ^{3}H-Thymidine and Allografting of Full-Thickness Skin Graft (M ± m)

Index studied	Time after injection of ^{3}H-thymidine				Thymidine not injected
	1 h	4 days			
			allografting		allografting
	control (skin)	control (skin)	skin surrounding graft	graft from mice not receiving ^{3}H-thymidine	graft from mice receiving ^{3}H-thymidine
Number of rows of cells in malpighian layer of epidermis	2,1±0,2	2,0±0,4	5,0±0,2	2,0±0,2	2,6±0,4
IMLE, %	47±12	60±16	54±6	Фон	61±17
Mean number of grains of silver above nucleus of epithelial cell	5,1±0,4	4,4±0,4	3,8±0,3	Фон	4,0±0,3
Mononuclear index	1,3±0,2	1,5±0,4	24±4	21±4	38±4
Number of labeled mononuclear cells/1000 epidermal cells	0,6±0,3	0,5±0,1	6,6±1,2	0,6±0,4	1,1±0,8
Mean number of grains of silver above nucleus of mononuclear cells	3,8±0,2	3,8±0,2	3,9±0,3	4,0±0,8	5,5±0,5
Leukocyte index	0	0	2,5±0,2	11±1	14±2
Number of mitoses per 1000 cells in malpighian layer of epidermis	2,0±0,6	1,5±0,3	6,6±0,98	0	4,0±1,1

wound as far as the graft bed in the region where it made contact with the skin graft along the edge of the incision in the dermis.

Changes were observed in the autoradiographic indices (in the skin surrounding the graft in the mice of experiments of series III, in the graft itself in the animals of series IV). Admittedly no significant differences were found between ILME in animals of all four series, but the number of grains of silver per labeled epidermal cell fell significantly in the skin surrounding the graft compared with the number in the skin of the mice of series I. About 25% of mononuclear cells infiltrating the epidermis surrounding the graft contained the label (on average four grains of silver per cell). No labeled cells were present in the graft in the animals of series III.

In the regenerating epidermis of mice the number of rows of cells in the malpighian layer thus is increased, i.e., the number of cells per unit area of skin surrounding the wound rises. Nevertheless, the fraction of labeled nuclei remains the same in the epidermis, although it would be expected to fall. The simplest explanation of this fact is by displacement of the surface epithelium into the region of the wound, for several investigations have shown that such migration is possible [8-10]. However, besides migrating, the keratinocytes can also proliferate, as shown by data in the literature [1, 3] and also the threefold increase in the number of mitoses in the regenerating epidermis. Proliferation of the keratinocytes is evidently linked also with a slight decrease in their level of labeling. Consequently, despite proliferation of the epidermis, the number of labeled nuclei and the total number of grains of silver in them were increased. At the same time, the proliferating epidermis was infiltrated by mononuclear cells, one-quarter of which contained label. It was perhaps their DNA which was the source of the additional ^{3}H-thymidine for the epithelial cells. Meanwhile, labeling values were the same for keratinocytes and mononuclear cells, which is in conflict with the view that DNA of dying mononuclear cells is re-utilized. However, this equality can easily be explained by an exchange of nuclear material between cells, as some investigators have postulated [6].

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