EFFECT OF INFLAMMATION ON ADHESIVE INTERACTION BETWEEN KERATINOCYTES

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KEY WORDS: adhesion; keratinocytes; inflammation; skin; dermoepidermal junction.

Dermatitis is often accompanied by excessive desquamation of corneal cells or even separation of the epidermis from the dermis. The cellular mechanisms of these phenomena have not been adequately studied.

In the investigation described below changes in adhesion both between keratinocytes in the epidermis and between the epidermis and dermis were studied in acute and chronic forms of inflammatory reaction.

EXPERIMENTAL METHOD

Noninbred male rats weighing 200-250 g were kept on an ordinary diet, one to a cage. Before injury, the animals were anesthetized by intraperitoneal injection of pentobarbital, 40 mg/kg body weight. The hair was removed in the interscapular region by depilation from an area of 24 cm^2. The animals were divided into two groups: experimental and control (at least five in each group). An acute inflammatory reaction was evoked in the skin by two methods: a) UV irradiation within the range of wavelengths 280-400 nm for 15 min by means of a type Élektronika-UFO-03-250 N source, placed 50 cm away from the part of the animal's body to be irradiated (adhesion was measured after the end of the 1st day; b) by a thermal burn, inflicted by applying a flat radiator, with area of 20 cm^2, kept at a constant temperature of 60°C, for 25-30 sec to the animal's dorsal region (adhesion was measured after 1.5-2.5 h). A chronic inflammatory reaction in the skin was induced by two daily applications of a solution of sodium dodecylsulfate (SDS) in a concentration of 7.5% for 21 days, whereas physiological saline was applied to the skin of animals in the control group (adhesion was measured with effect from 24 h after the last application). Adhesive interactions between keratinocytes were characterized by measuring two parameters reflecting the strength of coupling between the keratinocytes and the strength of the dermoepidermal junction. The strength of coupling of the keratinocytes in the epidermis (F, g/cm^2) was estimated with the aid of an "Epitest" adhesimeter ("Biotekh-Élektron," Moscow), by stripping off a thin (2-3 μ) layer of epidermis, glued to the end of a cylindrical steel probe 3 mm in diameter (MK-7M glue, rate of stripping 100 mm/min) [1]. The glueing and stripping procedure was repeated on the same area of skin 4 times, with the probe penetrating deep into the epidermis. The results obtained for the first stripping were disregarded, but for the subsequent three procedures they were analyzed after averaging: $\bar{F} = (\Sigma_{i=2}^{4} F_i)/3$. The strength of the dermoepidermal junction was estimated by means of an "Épitom" instrument ("Biotekh-Élektron," Moscow), by applying a negative pressure of −200 mm Hg to the skin [5]. Under the influence of this negative pressure the epidermis was separated from the dermis. The region of rupture of the dermoepidermal junction had the appearance of vesicles filled with exudate. The time of formation of these vesicles (t, min) was proportional to the strength of the dermoepidermal junction. The value of t in the control, which was 30-40 min, was always taken as 100%. The results were subjected to statistical analysis by nonparametric tests and by Student's t test.

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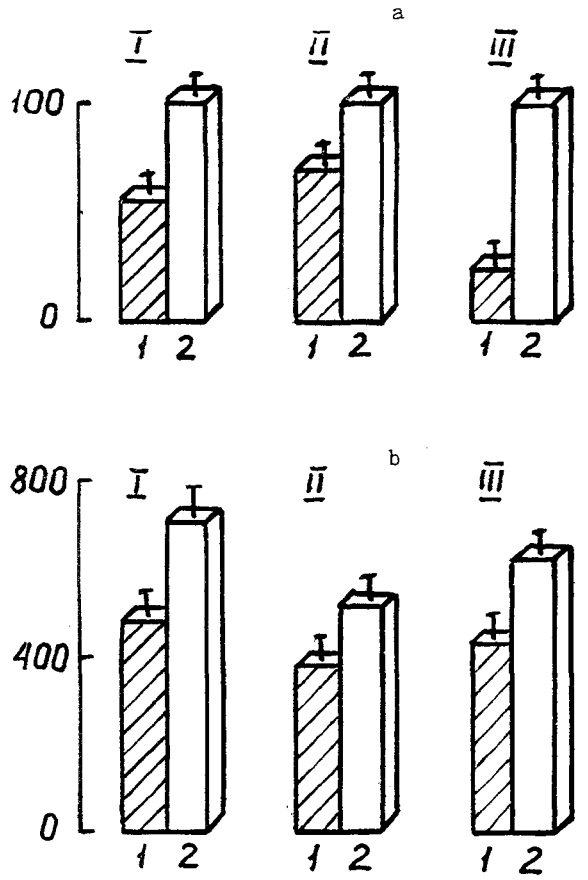

Fig. 1. Changes in adhesive interactions of rat skin keratinocytes during inflammatory reactions evoked by UV light (I), SDS (II), and heating to 60°C (III). a) Time (in min) of formation of epidermal vesicles (value of parameter in control group taken as 100%); b) strength of adhesion of keratinocytes (g/cm^2). 1) Experiment, 2) control.

EXPERIMENTAL RESULTS

The results given in Fig. 1a, b show that in all experiments, independently of the type of injury, the strength of coupling of the keratinocytes, like the strength of the dermoepidermal junction, decreased ($p < 0.05$). The decrease in the magnitude of adhesion, moreover, was more marked in the case of acute than of chronic inflammation. The greatest changes in the parameters studied were observed after thermal burning: the strength of linkage of the keratinocytes was reduced by 34%, whereas the strength of the dermoepidermal junction was reduced more than fourfold.

It can be considered that an inflammatory reaction in the skin is characterized, first, by weakening of connections between one keratinocyte and another, and between keratinocytes and the basement membrane; second, by nonspecificity of this reaction in response to different types of injury.

The effects observed probably reflect destructive changes in the skin.

Meanwhile, weakening of adhesion also evidently plays an adaptive role, facilitating the rapid desquamation of damaged cells and stimulating the process of cell renewal in the tissue.

We know that a frequent result of injury to the skin (for example, chemical, thermal, and radiation burns) is separation of the epidermis from the dermis, with the formation of blisters [4]. Similar destructive changes are observed in bullous diseases and some forms of allergic dermatitis [2-4]. The cellular mechanism of these changes is

evidently similar in all cases and consists of disturbance of adhesive interactions of keratinocytes, either among themselves or with the basal tissue membrane.

The high sensitivity of the chosen parameters to the destructive changes investigated suggests that the study of adhesive interactions between keratinocytes may prove to be useful both when studying the mechanisms causing these changes and when assessing the efficacy of therapeutic and preventive measures.

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QUANTITATIVE CHARACTERISTICS OF INTERACTION BETWEEN SERUM LIPOPROTEINS AND STEROID HORMONES

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The role of the blood lipoproteins (LP) in the transport of lipids [10], fat-soluble vitamins [11], xenobiotics [17], and thyroxine [4, 8] has recently been conclusively proved. Our previous results indicate that blood plasma LP can bind with and transport steroid hormones [1, 5, 6]. New, previously unknown properties of LP, determining their involvement in the regulation of intracellular metabolism, have been discovered. It has been shown that LP can penetrate into cells, take part in metabolism, and exert a regulatory influence [3]. Apoproteins are known to have coenzyme functions [9]. However, the role of the protein component of LP (apo-LP) in the body has been studied extremely inadequately. For instance, in order to understand the mechanisms of oriented transport and how certain hormones realize their effect, it is very important to study the possibility of their interaction with apo-LP.

In the present investigation, the glucocorticoid-binding capacity of the blood serum LP and of their protein components (apo-LP) was analyzed quantitatively by methods of fluorescent spectroscopy and equilibrium dialysis.

EXPERIMENTAL METHOD

Preparative isolation of LP from blood plasma was carried out by ultracentrifugation in KBr solution [12] on a "Beckman L-75" centrifuge with 75 Ti rotor. The very low density LP (VLDL, density under 1.006 g/cm^3), low density LP (LDL, density 1.006-1.063 g/cm^3), and high density LP (HDL, density 1.063-1.21 g/cm^3) thus obtained were dialyzed for 24 h against 0.15 M NaCl, pH 7.4, containing 0.005 M EGTA-Na_2 at 4°C. The LP were delipidized with a cold chloroform–methanol (2:1) mixture and then washed with ether. ApoA-1 was obtained by gel-filtration

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