CYTOHISTOLOGICAL MECHANISMS OF DEVELOPMENT OF MUCOSAL CANDIDIASIS AFTER TREATMENT WITH A STEROID-CONTAINING AEROSOL

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Systemic administration of corticosteroids (CS) is an important risk factor for the development of various forms of candidiasis [1, 5, 7, 8] which is linked with the general immunodepressive effect of these hormones. Meanwhile local application of CS to the mucous membranes in small doses which do not change the blood hormone levels and have no immunodepressive effect can also lead to the development of candidiasis. In particular, in the treatment of bronchial asthma with aerosols containing CS, the development of candidiasis of the mucosa of the mouth, pharynx, and larynx has been observed with a frequency proportional to the duration of administration of CS and their dose [2, 6, 9]. The writers have reproduced experimentally the development of candidasis of the oral mucosa following local application of CS and have studied some of its cytohistological mechanisms.

EXPERIMENTAL METHOD

Experiments were carried out on 62 male CBA mice weighing 15-20 g. The mouth was treated for 7-10 days with the drug Beclomet (from Orion Pharmaceutica, Finland) in an aerosol pack at the rate of five standard doses daily (each dose contained 50 μg of belcomethasone dipropionate). Infection with *Candida albicans* (strains 2565 and 991, isolated and identified at the All-Union Center for Deep Mycoses, Ministry of Health of the USSR) was then carried out by administration with the drinking water (10^6 blastopores/ml) for 1-3 days. The experimental results were evaluated histologically from 4 h to 7 days after infection; sections of the tongue and cheek, stained by the PAS reaction and with hematoxylin were analyzed, the state of the mold determined and the severity of the tissue reactions assessed. In some animals the effect of CS on adhesive activity of the fungal cells to epitheliocytes (EC) of the oral mucosa was studied before infection. This activity was expressed as the adhesion number (AN) — the mean number of fungal cells attached to one EC, and the adhesion index (AI), the relative percentage of EC with 10 or more adherent fungal cells [3, 10].

EXPERIMENTAL RESULTS

In control animals not receiving CS [9] or a suspension of the fungus [5] no histological changes were found in the mucosa of the tongue and cheek. On treatment of the mucosa with CS for 7 days and administration of the fungus for 1 day, 60% of the animals developed mycotic stomatitis. After treatment of the mucosa for 10 days and administration of the fungus for 1 and 3 days, lesions were found in 67% and 90% of mice, respectively. Histological analysis revealed the sequence of development of mycotic lesions, which were most marked on the dorsal surface of the tongue: during the first few hours after infection focal adhesion of the fungus to the surface of the mucosa took place, with the formation of growth tubes, which grew into the stratum corneum of the epithelium. The newly formed pseudomycelium (1-2 days) spreads within the stratum corneum without evoking any appreciable tissue reaction (Fig. 1). On the 3rd-7th day after infection the pseudomycelium invaded the malpighian layer, where it was destroyed by neutrophilic granulocytes, migrating into the epithelium (Fig. 2).

It is thus clear that differences between animals receiving CS and control mice began to appear in the earliest stages of interaction with the fungus: only in the former was

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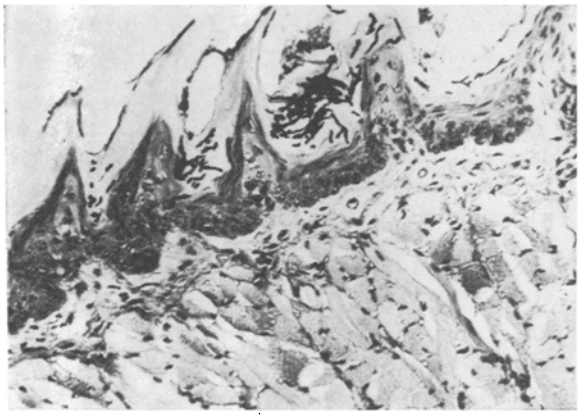

Fig. 1

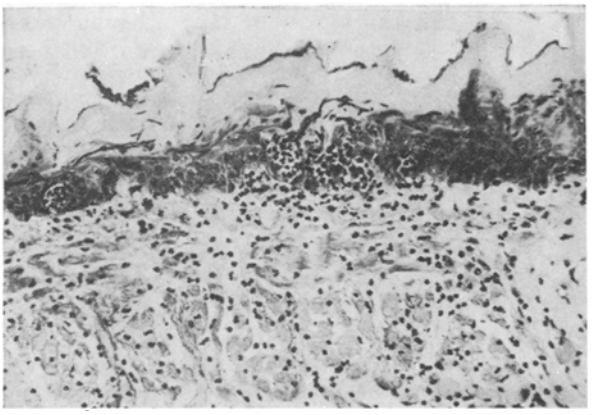

Fig. 2

Fig. 1. Spread of pseudomycelium of fungus within stratum corneum of mucosa of mouse tongue. Stained by PAS reaction and hematoxylin. 160 ×.

Fig. 2. Invasion of malpighian layer of epithelium of lingual mucosa by pseudomycelium of fungus; destruction of fungal cells by neutrophilic granulocytes migrating into epithelium; degenerative changes in muscle tissue of the organ. Stained by PAS reaction and hematoxylin. 160 ×.

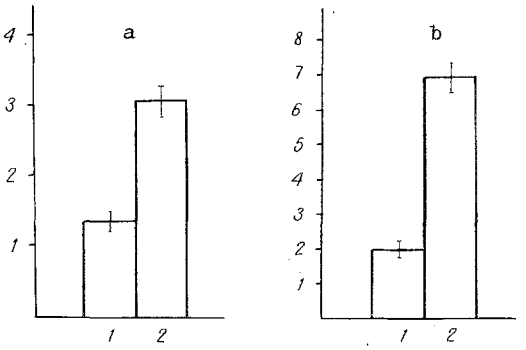

Fig. 3. Adhesive activity during interaction between *Candida* cells and epithiliocytes of control mice (1) and mice treated with corticosteroids (2). a) Adhesion number; b) adhesion index. Parameters (M ± m) determined 1 h after combined incubation at 28°C of equal volumes of fungal and epithelial cells (ratio 200:1). Parameters of animals of the experimental group differ from those in the control ($P < 0.01$).

effective adhesion of the fungal cells to the epithelium found in numbers sufficient to enable subsequent invasion. Changes in the adhesive properties of the epithelium were confirmed by the results of tests in vitro, showing an increase of adhesion under the influence of CS (Fig. 3). According to the results of cytologic analysis, the strongest adhesion (AN = 5.0 ± 0.4) was characteristic of EC with a nucleus, only a few of which (5%) were present in the preparation. Weaker adhesion (AN = 1.2 ± 0.2) was characteristic of keratinized EC, which constituted the overwhelming majority (95%). After injection of CS the number of EC with a nucleus increased to 10%, but this was responsible for only part of the increase in the mean AN, which was due to a greater degree to an increase in AN of the keratinized EC (AN = 1.8-0.2).

Characteristically, under the influence of CS not only the mean level of adhesion but, in particular, the number of EC with the highest adhesive properties changed, in agreement with the focal character of adhesion of the fungus and of its invasion observed on histological investigation. Differences in adhesion connected with the action of CS, observed in

vitro, were evidently intensified even more in vivo, where adhesion of the fungi prevents desquamation of the epithelium and their removal with food and saliva.

According to histological data CS have no marked effect on migration of neutrophilic granulocytes into a focus of information, and within the times studied they do not significantly inhibit their fungicidal mechanisms, thus leading to localization of the invading agent at the level of the epithelieum, followed by its destruction. In mucosal candidiasis induced in man by administration of Beclomet, the lesions also are relatively superficial in character, but they appear only after many weeks of use of the preparation [2]. A factor contributing to the comparatively rapid development of *Candida* infection in the present experiments was the larger doses of CS than are given to man, and the high concentration of fungal cells in the drinking water, many times greater than their concentration in human saliva (100 blastopores/ml [4]), in which *Candida* cells are often found in the form of saprophytes.

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IMPAIRED EPITHELIAL REGENERATION IN THE MUCOSA AS A FACTOR IN THE PATHOGENESIS OF ACUTE GASTRIC AND DUODENAL ULCER

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In the modern view the pathogenesis of acute gastric and duodenal ulcers is based on stress-induced ischemic damage to the mucosa, leading to a disturbance of cellular metabolism, to reduced formation and altered composition of the mucous, and to impaired reparative capacity of the epithelium [5, 6]. As a result the sensitivity of the mucosa, especially of the fundal part of the stomach, to bile acids and lysolecithin is increased and diffusion of hydrogen ions from the gastric contents (pH 1.0) into the interstitial fluid (pH 7.4) is intensified. Under normal conditions hydrogen ions diffusing into the tissue are neutralized by bicarbonate anions arriving from the blood or secreted by the parietal cells. If the mucosa is ischemic this does not happen and, as a result, cellular acidosis develops [2, 3, 5, 7]. Such cells are easily damaged by pepsin accumulating in the mucosa of the fundus of the stomach [4], as is confirmed by the protective action of its inhibitor in animals even at pH values of 1.3 [1].

Vincristine, widely used in clinical practice for the treatment of hemoblastoses, was used as a substance with direct action on cell regeneration processes. Binding with subunits of the protein tubulin, vincristine prevents the formation of microtubules of the spindle, so that divergence of the centrioles toward the poles of the cell is disturbed and mitosis

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