EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Proliferative Processes in the Epidermis of Patients with Atopic Dermatitis Treated with Thymodepressin

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Proliferative activity of the epidermis in skin biopsy specimens from patients with atopic dermatitis before and during therapy with thymodepressin (immunosuppressant) was studied by immunohistochemical method (by expression of Ki-67 antigen). The number of Ki-67-positive keratinocyte nuclei in atopic dermatitis considerably surpassed the corresponding parameter in intact skin (32.46±3.06% *vs.* 8.73±1.28%, $p<0.05$). Two 10-day courses of thymodepressin (0.1% solution, 1 ml intramuscularly) for 30 days reduced the number of Ki-67-positive keratinocyte nuclei to 20.78±3.36%. Clinical improvement was also achieved (sleep became normal, itching decreased, erythema and desquamation also decreased or disappeared). These findings suggest that disorders in keratinocyte proliferation are an important component in the pathogenesis of atopic dermatitis and confirm high efficiency of thymodepressin in the treatment of this condition.

Key Words: *atopic dermatitis; thymodepressin; Ki-67; proliferation*

Dysfunction of the immune system plays an important role in the pathogenesis of atopic dermatitis [6]. Atopic dermatitis resistant to routine therapy is effectively treated with cyclosporin, a potent immunosuppressor [5]. On the other hand, immunocorrective therapy including pyrogenal, thymogen, and epithalamine is also used [4]. In light of this, the use of thymodepressin, a nowel immunocorrector created at Peptos Research and Production Center by V. I. Deigin, seems to be promising in therapy of this condition.

Thymodepressin is a synthetic dipeptide consisting of D-glutamic acid and D-tryptophan residues. It is a mirror copy of immunoregulator thymogen (L-Gly-L-Trp). In contrast to thymogen, thymodepressin produces an immunosuppressive effect. Treatment of intact bone marrow with thymodepressin markedly suppresses colony formation in the spleen, which is restored after injection of intact thymocytes [8]. Thymodepressin inhibits proliferation of hemopoietic precursor cells and stimulates granulocyte and lymphocyte apoptosis [1,2]. Thymodepressin is effective in the therapy of psoriasis.

MATERIALS AND METHODS

Fifteen patients with neurodermatitis were examined. Biopsy specimens of involved skin (lower, upper, and median part of forearm) were collected before and after therapy at 8.00-10.00. Thymodepressin (0.1% solution, 1 ml) was injected intramuscularly at 8.00-10.00 for 30 days (2 courses, 10 injections per course, with 10-day interval). Control specimens of normal skin were collected from 20 patients subjected to planned interventions for hernias (the specimens were col-

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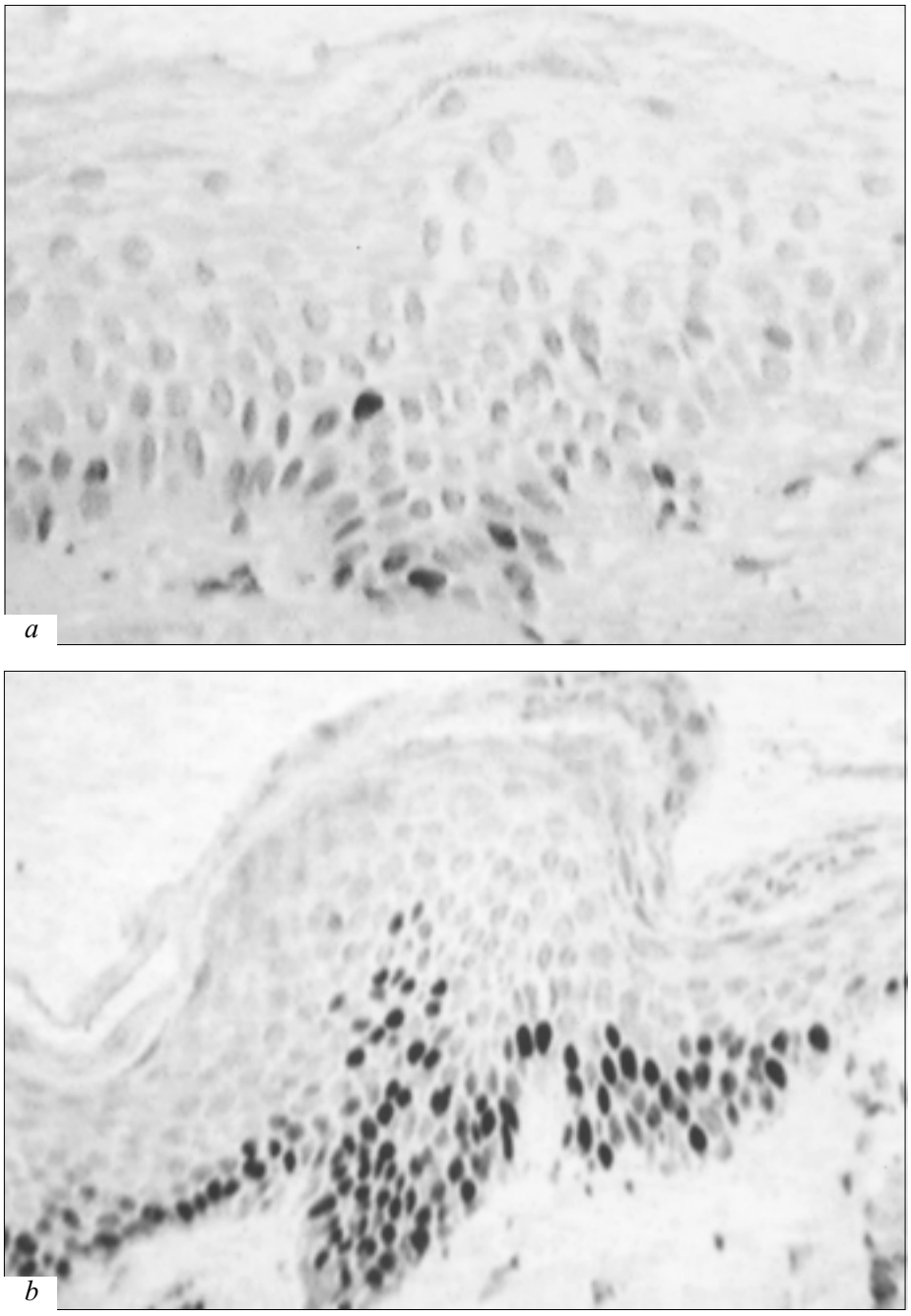

Fig. 1. Expression of Ki-67 antigen in intact skin keratinocytes (*a*) and keratinocytes from patients with atopic dermatitis (*b*), ×70. Indirect immunohistochemical method with diaminobenzidine visualization and hematoxylin post-staining.

lected at the median line of the abdomen, from the right and left ileac areas). The specimens were fixed in 10% neutral formalin in phosphate buffer for 24 h and embedded in paraffin. The sections (7 μ) were transferred to slides treated with 0.01% poly-L-lysine (Sigma). In order to restore the antigenic structure after deparaffination, the sections were treated with Target Retrieval Solution (DAKO) in the recommended concentration for 20 min at 95°C, after which the sections were treated with antibodies to protein Ki-67, second antibodies, streptavidin (DAKO), and visualized with 0.1% diaminobenzidine. The sections were post-stained with Lily—Mayer hematoxylin. The number of positively stained keratinocyte nuclei was evaluated in percent of the total number of nuclei in the regeneratory zone of the epidermis after examination of at least 100 nuclei in each biopsy specimen.

The results were statistically processed using Student *t* test. The differences between the groups were considered significant at $p<0.05$.

RESULTS

The number of Ki-67-positive nuclei in normal skin was 8.73±1.28%, which agrees with published data that 4-12% nuclei in normal skin express Ki-67 antigen [7,9,10]. The label was located mainly in the basal and parabasal layer of keratinocytes. In patients with atopic dermatitis the index of proliferating cells increased to 32.46±3.06%. Activation of proliferative processes in the skin in atopic dermatitis was revealed previously [3] by flow cytometry, but this method does not allow direct evaluation of proliferation process in this disease. In atopic dermatitis the zone of Ki-67 expression was located not only in the basal layer, but also in the prickly layer. Solitary labeled nuclei were seen in the granular layer (Fig. 1).

Thymodepressin therapy provided marked clinical improvement (sleep normalized, itching was partially alleviated, erythema decreased and in some cases disappeared). Desquamation decreased in the course of treatment and later disappeared.

Clinical improvement was paralleled by inhibition of hyperactivated repair processes. The percentage of nuclei labeled with antibodies to Ki-67 protein decreased to 20.78±3.36% ($p<0.05$).

Hence, abnormal proliferation of keratinocytes is an important component of the pathogenesis of atopic dermatitis. This is confirmed by an increase in Ki-67 expression in this disease and significant reduction of this parameter after clinical improvement.

These results demonstrate high efficiency of thymodepressin in atopic dermatitis.

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