Immunomorphological Study of Langerhans Cells in the Skin of Patients with Atopic Dermatitis

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Immunomorphological study of Langerhans cells in affected skin of patients with moderate exacerbation and remission of atopic dermatitis revealed an increase in numerical density (compared to healthy volunteers) and change in morphological characteristics and topography of these cells.

Key Words: *atopic dermatitis; skin; Langerhans cells; immunomorphological and morphometric study*

The role of dendritic cells (Langerhans cells, LC) in the pathogenesis of atopic dermatitis (AD) is poorly understood. During AD, the major function of LC (antigen binding and presentation to T lymphocytes) is realized not only in T-dependent zones of lymph nodes, but also in the epidermis and dermis [7]. They carry surface Fcε receptors for IgE [8]. LC have exhibit secretory activity and produce interleukins, colony-stimulating factors, interferon-γ [2,4,12], and adhesion factors [3,6,7], thus modulating the development of inflammatory processes. LC have a variety of functions, which suggests the possibility for changing some morphological properties of cells at different stages of AD.

The relationship of typical location and manifestations of skin rash with structural and morphological characteristics of affected areas in AD remains unknown.

Here we performed an immunomorphological study and evaluated quantitative dynamics of LC in affected skin of AD patients.

MATERIALS AND METHODS

We examined the patients (14-36 years old) with erythematous-squamous AD and lichenification of different severity. There were 2 groups of patients with moderate exacerbation (group 2, n=20) and remission of the disease (group 3, n=14). Skin samples from healthy volunteers served as the control (group 1, n=7).

All patients were informed about the trial. Informed consent was obtained from the patients. Informed consent for skin microbiopsy was obtained from parents of patients younger than 18 years.

Tissue samples were isolated from the cubital fossa (typical location of abnormal elements in the skin during AD). For light microscopy, the samples were fixed in 10% aqueous solution of neutral formalin, dehydrated in alcohols of increasing concentrations, and oriented in paraffin blocks for preparing tissue sections perpendicular to the skin surface. Sections (5 μ) were stained with van Gieson hematoxylin and eosin. Immunohistochemical study of deparaffinized sections and antigen demasking in a microwave oven were performed for visualization and assay of LC.

The biomolecular marker (Novacastra) Lag^+ was detected (type II Ca^{2+}-dependent lectin expressed only by LC and involved in the formation of Birbeck cytoplasmic granules) [11]. Visualization was performed using an Envision detection system (Dako). Skin samples were incubated with the antibodies at room temperature for 60 min. After incubation, the sections were stained with hematoxylin.

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The Lag^+-positive reaction resulted in brown staining of LC cytoplasm.

We estimated the numerical density of LC (per 1 mm^2 skin section) [1] and number of cytoplasmic processes (per dendritic cell). The size of cells corresponded to the mean diameter of LC bodies (dendritic and round-shaped cells). The length of processes was not taken into account. The topography of LC was evaluated relative to the basal membrane and epidermal layers.

The significance of differences between the means was estimated by Student's *t* test. These differences were significant at $p<0.05$.

RESULTS

The numerical density of epidermal LC in group 2 patients was by 35.8% higher than in group 1 volunteers (Table 1). LC were regularly distributed in the skin of healthy volunteers. They were located in the basal and lower regions of the prickle-cell layer in the stratified squamous epithelium and had no intercellular junctions. By contrast in affected skin of patients with AD exacerbation, LC were displaced to the surface region of the prickle-cell and granular layers. LC were located irregularly and arranged in clusters of 3-12 cells (per field of view). They alternated with regions free from these cells.

The size of LC in skin samples from group 2 patients was 18% lower compared to that in group 1 volunteers (Table 1). This parameter significantly varied in biopsy specimens from each patient (15.6-20.8 μ). We showed that 22.6% cells of the total number of LC were characterized by the smallest size, round shape, and absence of typical cytoplasmic processes. Moreover, 77.4% LC had dendritic shape and vertically oriented processes. Some processes penetrated the dermis and came into contact with the wall of blood capillaries. Individual lateral processes were shortened and did not anastomose with each other. In the epidermis of group 1 volunteers, 93.6% LC had dendritic shape and lateral cytoplasmic processes that were in contact with each other. The mean number of LC processes in healthy volunteers was higher than in group 2 patients (Table 1).

The numerical density of epidermal LC in group 3 patients was lower than in group 2 patients (by 24.7%), but higher than in group 1 volunteers (by 15%, Table 1). The majority of cells (90.5%) had dendritic shape and was located in the basal, lower, and middle regions of the prickle-cell layer. The size of LC and number of cell processes in group 3 patients were similar to those in group 1 volunteers (Table 1).

These data suggest that the skin of AD patients includes LC in 2 functional states, which determines morphological differences between these cells.

The number of LC in the surface layer of the epidermis increased during exacerbation of the disease. These cells were characterized by small size, round shape, and absence of cytoplasmic processes. The increase in the number of LC during exacerbation of AD is probably associated with the recruitment of new dendritic cells from bone marrow precursors [3,5,9].

Another functional type of LC was immunophenotyped in the skin of healthy volunteers and patients with exacerbation or remission of AD. They were characterized by dendritic shape and presence of cytoplasmic processes. Exacerbation of AD was accompanied by changes in the direction of processes (from lateral to vertical). These cells were mainly located in the basal and prickle-cell layers of the epidermis. Published data show that class II major histocompatibility complex molecules (HLA-DR) are expressed on the outer surface of the cell membrane in cytoplasmic processes of LC [3,10]. LC of this type are probably responsible for antigen presentation to $CD4^+$ T lymphocytes [3].

Our results indicate that exacerbation of AD is accompanied by an increase in the numerical density of LC in affected skin and displacement of cells to the surface layer of the epidermis. The existence of LC in different functional states is associated with variations in immunophenotypic characteristics, including the size, shape, and presence and direction of cytoplasmic processes.

TABLE 1. Morphometric Study of Epidermal LC in Human Skin from the Cubital Fossa (*M*±*m*)

Group	Numerical density of LC per 1 mm^2 skin	Size of LC, m	Number of cytoplasmic processes per LC
1	300.58±3.50	21.20±0.26	2.600±0.045
2	468.26±6.20*	17.34±0.20*	2.100±0.062*
3	352.63±4.1*+	20.58±0.18*+	2.530±0.035+

Note. $p<0.05$: *compared to group 1; +compared to group 2.

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