IMMUNOHISTOCHEMICAL ESTIMATION OF SKIN CONDITION IN THE PATIENTS WITH ATOPIC DERMATITIS IN THE COURSE OF TREATMENT

Based on immunomorphological method it has been revealed dominancy of skin associated T-lymphocytes (CD45RO+) over T-lymphocytes expressed by receptors (CD45RA+) in the patient’s skin suffering from atopic dermatitis. The result can be used for therapeutic correction of the specified changes with use of nicergoline (sermion).

Keywords: Atopic dermatitis, immunomorphology, T-lymphocyte, nicergoline.

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Introduction

Nowadays atopic dermatitis (AD) is considered to be a systemic disease, where pathologic process involves many organs and systems of an organism (Toropova et al., 1997; Sergeyev, 2002).

Basically, the disease develops within the first months of life, manifests by chronic allergic inflammation of cutaneous covering with a recurrent flow, which is difficult to be treated. In this regard, there is a need for further study of predisposing and causal-significant factors, pathogenic mechanisms, and peculiarities of clinical presentations, introduction of modern diagnosis methods into clinical practice, treatment and prevention of atopic dermatitis (Protsenko, 1998).

The development of immunology in the last years helped to find out anomalous regulation of cytokines profiled as T-helpers within the 1 and 2 order, increase in number of extremely sensitive IgE receptors in the cells of Langerhans, circulation of IgE and its presence in skin. But the evidence of immunohistochemical changes in the skins of patients suffering from atopic dermatitis is uncommon and contradictory (Leung, 1999; Butov and Podolich, 2005).

Therapy of any illness might be successful only after defining the fundamental mechanisms of its development; therefore, the studies aiming to further understanding the development of pathogenetic factors of AD make possible to open new potential targets to therapeutic intervention.

The paper aims to discuss immunohistochemical changes during the course of treatment in the skins of patients suffering from atopic dermatitis.

Materials and methods

There were observed 35 patients suffering from atopic dermatitis and 35 healthy volunteers. The biopsy of skin in the patients was carried out by the author. Histological and immunohistological study was conducted under the agreement with a laboratory “Consultant Biotech”. Immunohistochemical analysis was done on the sections with paraffin block tumours, which are dedicated to a standard morphological study. The sections of tissues were fastened onto special glass slides which have positive electrical discharge (SuperFrostPlus, Menzel-Glaser, Germany). The paraffin sections were dried out at 37ºС throughout 12 hours. Before the painting procedure the glass slides with sections were put into thermostat (at +56ºС) for 30 minutes. The paraffin sections were de-embedded and dehydrated in accordance with a standard method (3 alternations of xylene and 3 alternations of ethyl alcohol for 10 minutes). The disclose of antigens was
held in a liquid of target restoration buffer “Dako”, Denmark (pH=6.0 or hH=9.0) with use of a water bath at 95-98°C in 20-40 minutes. Then, glasses were cooled at room temperature within 15-20 minutes not taking the sections out from the containers with the buffer. The characteristic of antibodies used in work and conditions of decamouflage are shown in Table 1. With a purpose to block endogenous peroxidase, the sections were processed with a 3% solution of hydrogen peroxide for 10 minutes. Incubation with primary antibodies was held at room temperature within 30 minutes. Incubation with secondary antibodies EnVision™+System/HRP (DAB+) or LSAB®+kit (Dako, Denmark) was held according to the instruction. To visualize the immunohistochemical reactions there was used diaminobenzene, which was controlled under a microscope for 5-10 minutes. The sections were being painted with a hematoxylin of Mayer (DAKO, Denmark), dehydration was conducted in three alternations of ethyl spirit and in 4 alternations of xylene with the subsequent conclusion into balm.

TABLE 1. CHARACTERISTICS USED FOR IMMUNOHISTOCHEMICAL RESEARCH OF ANTIBODIES

<table>
<thead>
<tr>
<th>Name of antibody</th>
<th>Titre</th>
<th>Clone</th>
<th>Decamouflag, time</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 45</td>
<td>RTU</td>
<td>PD7/26  and 2B11</td>
<td>pH=6.0; 20 min.</td>
<td>Dako (Denmark)</td>
</tr>
<tr>
<td>CD 45RA</td>
<td>1/50</td>
<td>4RB5</td>
<td>pH=6.0; 20 min.</td>
<td></td>
</tr>
<tr>
<td>CD45RO</td>
<td>RTU</td>
<td>UCHL1</td>
<td>pH=6.0; 40 min.</td>
<td></td>
</tr>
<tr>
<td>CD15</td>
<td>1/25</td>
<td>C3D-1</td>
<td>pH=9.0; 20 min.</td>
<td></td>
</tr>
<tr>
<td>CD68</td>
<td>RTU</td>
<td>KP1</td>
<td>pH=6.0; 20 min.</td>
<td></td>
</tr>
</tbody>
</table>

Estimation of results in colouring was carried out with use of light microscope Nikon Eclipse E200 (Japan) zoomed to x100, x200, x400.

Complex immunohistochemical research included defining CD68 +, CD45RO +, CD45RA +, CD15 + cells in epidermis, in a zone of dermo-epidermal borders and in a perivascular infiltrates of an affected skin. Markers of a cellular differentiation are characterized as follows:

- CD68 - a marker of histiocyte and macrophages. CD68 is expressed in a cytoplasm of monocyte, macrophages, osteoclast and corpulent cells. It reacts with myeloid cells as well. To the list of CD68-positive can be included activated T-cells, subpopulation of mature B-cells, epithelium (in cytoplasm);
- CD45RO - low-molecular isoform total leukocytic antigen (LCA/CD45) which is associated with T-lymphocytes. CD45RO comes to light in the majority thymocytes, in the maturely activated T-cells, in the subpopulation based on T- lymphocytes (both in CD4+ and CD8+). Antibody UCHL1 reacts with granulocytes and monocytes, but does not react with NK-cells and normal B-lymphocytes. T-cellular lymph is characterized by performance of CD45RO;
- CD45RA - is typical for normal and neoplastic cells, which exposes in the germinal centers of lymphoid follicles and amphicyte zones. It also defines lymphocytes located in a derma of skin;
- CD15 - an antigen of granulocytes, monocytes, epithelial cells which are involved in processes of phagocytosis. The CD15 is also shown in tumorous cells of some T - and B cellular non-Hodgkin's lymphoma;
- CD 45 - a general leukocytic antigen

Determination of the number of positive cells in epidermis and in the borders of dermo-epidermic zone was conducted in 40 fields of vision zooming up to 400. To esteem the maintenance of cells in the infiltrates of skin and in 5 perivascular infiltrates, the 1000 cells were chosen randomly among which calculation of quantity of cells which were marked with a specific antibodies was done. Then, for every biopsy material the average number
was counted depending on the maintenance of the cells. Patients were given, in combination with therapy, nicergoline (sermion) to the center intradermal.

Results and discussion

Before the treatment patients suffering from atopic dermatitis had a dominance of skin-related T-cells CD45RO + (Figure 1) and T-lymphocytes with skin lymphocytic antigen – “naïve” CD45RA + (Figure 2) in the composition of perivascular infiltrate. The number of skin-related T-cells (CD45RO +) has made 66.7±4.2, activated T-lymphocytes with skin lymphocytic antigen (CD45RA +) was 19.3±1.4 out of 100 cells of infiltrate. Only few cells were found in a basal layer of epidermis, the number of CD45RO + was 4.3±1.2 cells, and CD45RA + 0.3±0.06 out of 100 keratinocytes of a basal layer (Figure 1) (Table 2).

Table 2. The content of populating cells in epidermis and in perivascular infiltrates of skin in both patients suffering from atopic dermatitis and healthy volunteers, who were receiving sermion (M+M)

<table>
<thead>
<tr>
<th>Cell population</th>
<th>Healthy volunteers (n = 35), cells</th>
<th>Patients suffering from atopic dermatitis Before treatment (n = 35), cells</th>
<th>Patients suffering from atopic dermatitis After the treatment (n = 35), cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45</td>
<td>6.5±0.55</td>
<td>72.58±0.02</td>
<td>22.05±0.01</td>
</tr>
<tr>
<td>CD45RO</td>
<td>0</td>
<td>0.28 ±0.06</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>CD45RA</td>
<td>0.14±0.09</td>
<td>4.3 ±1.2</td>
<td>1.4±0.5*</td>
</tr>
<tr>
<td>CD68</td>
<td>6.1±0.55</td>
<td>53.0 ±7.6</td>
<td>16.2±4.7*</td>
</tr>
<tr>
<td>CD15</td>
<td>0</td>
<td>10.2 ±2.5</td>
<td>3.03±0.21</td>
</tr>
</tbody>
</table>

Note: P<0.05 - comparative performance of the level of statistical relevance of patients suffering from atopic dermatitis before and after the treatment.

Figure 1. Highly immunohistochemical expression of CD45RO in the derma of skin and in epidermis before the treatment. X400.

Figure 2. Immunohistochemical expression of CD45RA in the derma of skin and in epidermis before the treatment. X400.
Total (epidermis + derma) number of skin related T-cells (CD45RO +) in the skin of patients suffering from atopic dermatitis before treatment has made 66.9±4.2, T- lymphocytes with skin lymphocytic antigen (CD45RA +) - 23.6±2.3 cells which for 6 and 5 times surpass similar performances in the skins of healthy people (p<0.001). Whereas the number of CD45RO+ T-lymphocytes in a skin of patients excel more than 1.5 times the number of CD45RA+ T- lymphocytes. The comparative analysis has shown that the number of T- lymphocytes, which express receptors into skin (CD45RA) makes only 30% from the total number skin related T- lymphocytes.

In an inflammatory infiltrate there have been found out lymphoid elements which are also positive to CD45 (Figure 3), CD68 (Figure 4) and CD15 (Figure 5). Derma-epidermal connections and part of acanthaceous layers were also infiltrated with these elements with migration focuses to neutrophils in order to find in epidermis. Immunohistochemical research has shown that inflammatory infiltrates basically consisted from CD45RO+, partially from CD45RA +, neutrophils (CD15) and CD68+ histiocyte.
FIGURE 7. IMMUNOHISTOCHEMICAL EXPRESSION OF CD45 IN THE DERMA OF SKIN AND IN EPIDERMIS AFTER THE TREATMENT. X400
Light perivascular infiltration with lymphoid elements

FIGURE 8. IMMUNOHISTOCHEMICAL EXPRESSION OF CD45RO IN THE DERMA OF SKIN AND IN EPIDERMIS AFTER THE TREATMENT. X400
Single lymphoid elements are seen which are around the vessels

FIGURE 9. IMMUNOHISTOCHEMICAL EXPRESSION OF CD45RA IN THE DERMA OF SKIN AND IN EPIDERMIS AFTER THE TREATMENT. X400
Single lymphoid elements are seen which are around the vessels

FIGURE 10. IMMUNOHISTOCHEMICAL EXPRESSION OF CD68 IN THE DERMA OF SKIN AND IN EPIDERMIS AFTER THE TREATMENT. X400
Single lymphoid elements are seen which are in the derma of skin and in the lower layers of epidermis

FIGURE 11. IMMUNOHISTOCHEMICAL EXPRESSION OF CD15 IN THE DERMA OF SKIN AND IN EPIDERMIS AFTER THE TREATMENT. X400
In the derma of skin there is almost complete vanishing of CD15 positive neutrophils. There is a moderate expression in basal layer of epidermis.

Single lymphoid elements are seen which are in the derma of skin and in the lower layers of epidermis.

Level of an expression of a marker of histiocytes and macrophages (CD68) in the skins of patients was high both in epidermis and derma, in comparison with skins of healthy people considerably prevailed in epidermis - 53,0±7,6 cells, in derma 13,6±2,8 cells (p <0,001). The same dependence was highlighted at expression of CD15, the marker of granulocytes. So, the level of an expression of this marker was high before the treatment, both in epidermis (10,2±2,5) and in derma (15,5±2,8), and has considerably decreased after the treatment with sermion up to 3,03 in epidermis and 0,3 - in derma. Though there has been highlighted direct correlational dependence between expressions of receptors macrophages and histiocytes (CD68) and the maintenance of skin related T-lymphocytes (CD45RO + and CD45RA) (the correlation factor (r) between CD68 + and CD45RO+cells has made 0,5, between CD68 + and CD45RA + cells- 0,7) in a skin.

**Conclusion**

Thus, it is shown that atopic dermatitis proceeds against the disbalance of subpopulation structure of T-lymphocytes, occurrences of granulocytes and macrophages which develop a number of physiologically active substances which cause immune changes, have certain stages and they are basis of a clinical demonstration of diseases which have probability of transition into T-cellular lymphoma of skin.

In the process of work the immunomorphology method has revealed dominancy of skin associated T-lymphocytes (CD45RO+) over T-lymphocytes expressed by receptors (CD45RA+) in the patients skins' suffering from atopic dermatitis which can be used to carry out selective, additional to the basic treatment, therapeutic correction of the given changes.

After the treatment with application of sermion we have found out trustworthy decrease of all investigated indicators in both samples of skin (Table 2) that testifies of their pathogenetic importance in atopic dermatitis.

Thus, in the process of work the immunomorphology method has revealed dominancy of skin associated T-lymphocytes (CD45RO+) over T-lymphocytes expressed by receptors (CD45RA+) in the patient's skin suffering from atopic dermatitis which can be used for therapeutic correction of the given changes with nicergoline.

**References**


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