T-CELL SUBPOPULATIONS IN LESIONS OF ORAL LICHEN PLANUS

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Abstract

Aim of the study: To determine the distribution and frequency of T(CD3) cells and cell subpopulations in tissue specimens of erosive lichen planus (EOLP).

Material and Method: Tissue specimens from buccal mucosa were taken from 14 healthy individuals – control group (CG) – and 17 subjects with EF-OLP. Applying monoclonal antibodies, T(CD3) cells, T cell subpopulations, The CD4, CD8 and CD4/CD8 ratios in both groups were determined. Cells in the epithelium and lamina propria were quantitatively and qualitatively determined in both groups. Data were analysed using the Student’s t-test.

Results: There were 5.95 ± 2.12% T(CD3) cells in the control group as against 9.80 ± 4.04% in the examined group (p < 0.001). The distribution of T(CD3) cells in the lamina propria was 25.35 ± 12.04% in the examined group compared to the control group (p < 0.001). There were 3.45 ± 2.05% CD4 epithelial cells in the control group and 4.00 ± 1.95% in the examined group (p < 0.4). There were 2.50 ± 1.8% CD8 cells in the control group and 5.80 ± 3.72% in the examined group (p < 0.001). The CD4/CD8 ratio was 0.51 ± 0.12% in the examined group and it was evidently reduced in comparison with the control group. An increased distribution of CD4 cells (10.30 ± 7.60%) and CD8 cells (15.05 ± 5.20%) in the lamina propria compared with the epithelium was observed in the examined group (p < 0.001). The CD4/CD8 ratio in the epithelium was 0.51 ± 0.12% as against the ratio of the lamina propria, which was slightly increased (0.68 ± 0.48%) with a low statistically significant difference (p < 0.05).

Conclusion: Differences in the distribution of T-lymphocyte subsets between the control and examined groups were found (p < 0.001). An increased distribution and frequency of CD4 and CD8 cells in the lamina propria was observed. These were predominantly located in the sub-basal region of the stratum papillare and rarely seen in the intra-epithelial region.

Key words: erosive lichen planus, oral lichen planus, T(CD) cells, T-cell subpopulations, CD4 lymphocytes, CD8 lymphocytes.

Introduction

Oral lichen planus (OLP) is a chronic mucosal condition that is commonly encounte-red in dental practice. The heterogenic clinical finding is manifested on the buccal mucosa as white plaque, papules, white striations, eryt-
hema, erosions, ulcerations or blisters. Although modern science and medicine have modern methodology at their disposal, the etiology of oral lichen planus is still unknown. It is believed that lichen planus is an abnormal immune response in which epithelial cells are recognized as foreign, changing the antigenicity of the cell surface [1]. Roopashree [2] explains pathogenetic dilemmas with both antigen-specific and non-specific mechanisms. Antigen-specific mechanisms include antigen presentation by basal keratinocytes and antigen-specific keratinocytes being killed by CD8 (+) cytotoxic T-cells. The other, non-specific mechanisms include mast cells, thus causing cell degranulation and metalloproteinase activation in OLP lesions.

Pathohistological findings of liquefactive degeneration of the basal cells with abundant lymphocyte infiltrate and hyperkeratosis have led many researchers to believe that the principal and the so-called initial defect is in the epithelium [3]. However, over the past years research has been directed to submucosal infiltrates where the role of mononuclear infiltrate is an important step in resolving the pathogenetic mechanisms of the disease. With reference to this, Hasseus [4] confirmed the immunological background of erosive lichen planus and Ivanova [5] in her study proved the immune-mediated mechanisms in the pathogenesis of this disease. T-cell accumulation in the superficial layers, basement membrane disruption, as well as intracellular T-cell migration, are due to combined specific and non-specific mechanisms [2]. Motivated by the numerous earlier investigations into a possible immunopathological genesis of this disease, which are still not completed and are decidedly contradictory, we have set the aim of our study: to determine the distribution and frequency of T(CD3) cells and cell subpopulations in tissue specimens of EOLP.

Material and Methods

For the realization of our aim we examined 17 patients of different sex and age diagnosed with oral lichen planus, who were treated at the Clinic of Oral Pathology and Periodontology of the Faculty of Dentistry in Skopje. The study did not include patients with cutaneous manifestation or those who, in addition to cutaneous manifestation, had oral manifestation. Diagnosis was made on the basis of a thorough history, objective clinical finding and pathohistological verification.

Oral mucous specimens were obtained by biopsy, which was performed in sterile conditions, under local anesthesia. Biopsies were taken from the site of the lesion, that is, exclusively from buccal mucosa (1 cm in width and length), and from the deep epithelium as well as a segment from the lamina propria. The specimen was put in a sterile P-P PBS and passed to the Department of Nephrology, where it was frozen and then examined.

T(CD3) lymphocytes and subpopulations (CD4 and CD8) were determined in all patients with oral lichen planus.

Determination of CD markers in the biopsy specimens was done at the Clinic of Nephrology of the Medical Faculty in Skopje. Tissue specimens taken from patients with erosive lichen planus were treated with monoclonal antibodies and following the staining procedure the results were read on a microscope and were compared with those of the control group.

The control group consisted of 14 healthy individuals and biopsy specimens of their healthy buccal mucosa were taken. All the patients in the control group were healthy, without any intercurrent illnesses. They paid a visit to dental surgeons for surgical extirpation of impacted eight teeth. During surgery biopsy specimens from buccal mucosa were taken and later treated with monoclonal antibodies.

The results obtained were compared with those of the control group. All the results were statistically analysed by using the Student’s t-test for significance of differences.

Results

Table 1 presents the values of tissue distribution of T(CD3) in the epithelium and lamina propria in both the control and examined groups. T(CD3) cells were found in 5.95 ± 2.12% of control subjects as against 9.80 ± 4.04% in the examined patients (p < 0.001). Comparing the distribution of T(CD3) in the lamina propria between the control group and patients with oral lichen planus, an evident increase of T(CD3) lymphocytes in the sub-basal layer was detected in the examined group.
The T(CD3) value in the control group was 6.95 ± 4.05% (p < 0.001), (Fig. 1).

Table 2 illustrates tissue distribution of T-lymphocyte subsets (CD4, CD8) and their ratio in the epithelium of both the control and examined groups. CD4 cells (helper/inductor) in the epithelium of the control group were found in 3.45 ± 2.05% versus 4.00 ± 1.95% of the examined group. Statistical analysis showed no significant difference (p < 0.4). CD8 (suppressor/cytotoxic) cells were found in 2.50 ± 1.80% of control subjects and in 5.80 ± 3.72% of examined patients. There was a significantly high statistical difference between these two groups (p < 0.001). On the other hand, the CD4/CD8 ratio was 0.51 ± 0.12% in the examined group and it was evidently reduced in comparison with the controls (1.38 ± 1.02%). Thus, there was a highly significant difference between the groups (p < 0.001).

Values of T-lymphocyte subsets in the lamina propria of control and examined subjects are presented in Table 3. All CD-markers in the lamina propria had increased values in the examined group. CD4 cell values were 10.30 ± 7.60% in the examined group and 4.45 ± 2.05% in the control group. The CD8 cell mean value was evidently increased in the examined group (15.05 ± 5.20%) in comparison with the control group (2.50 ± 1.80%). Statistical analysis showed a highly significant difference of values between the two lymphocyte subpopulations (p < 0.001), (Fig. 2).

Table 1

<table>
<thead>
<tr>
<th>%</th>
<th>Epithelium</th>
<th>Lamina propria</th>
<th>Epithelium</th>
<th>Lamina propria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group n = 14</td>
<td></td>
<td></td>
<td>Examined group n = 17</td>
<td></td>
</tr>
<tr>
<td>x</td>
<td>5.95</td>
<td>6.95</td>
<td>9.80</td>
<td>25.35</td>
</tr>
<tr>
<td>SD</td>
<td>2.12</td>
<td>4.05</td>
<td>4.07</td>
<td>12.04</td>
</tr>
<tr>
<td>Se</td>
<td>0.56</td>
<td>1.08</td>
<td>0.98</td>
<td>2.92</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>3.09</td>
<td>5.30</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>***</td>
</tr>
</tbody>
</table>

Figura 1 – T(CD3) cells in epithelium and lamina propria of buccal mucosa in erosive oral lichen planus

The CD4/CD8 ratio was 1.78 ± 1.12% in the control group, whereas in the examined group this ratio was significantly reduced (0.68 ± 0.43%). There was a statistically significant difference between these two groups.
Table 2

**Tissue distribution of T(CD3) lymphocyte subsets in epithelium of control and examined subjects**

<table>
<thead>
<tr>
<th>%</th>
<th>Control group n = 14</th>
<th>Examined group n = 17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD4</td>
<td>CD8</td>
</tr>
<tr>
<td>χ</td>
<td>3.45</td>
<td>2.50</td>
</tr>
<tr>
<td>SD</td>
<td>2.05</td>
<td>1.80</td>
</tr>
<tr>
<td>Se</td>
<td>0.54</td>
<td>0.48</td>
</tr>
<tr>
<td>t</td>
<td>0.74</td>
<td>2.94</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.4</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

The comparison of T-lymphocyte subpopulation between the epithelium and lamina propria is given in Table 4. An increased distribution of both CD4-cells (10.30 ± 7.60%) and CD8-cells (15.05 ± 5.20%) was observed in the lamina propria of examined patients. Mean values of these CD-markers were lower in the epithelium and reached 4.00 ± 1.95% for CD4 and 5.80 ± 3.72% for CD8. Therefore, there was a highly significant statistical difference between CD4 and CD8 values (p < 0.001). The CD4/CD8 ratio in the epithelium was 0.51 ± 0.12% as against the ratio in the lamina propria, which was slightly increased and reached 0.68 ± 0.48% revealing a low statistically significant difference between the values (p < 0.05) (Table 4).

Table 3

**Tissue distribution of T(CD3) lymphocyte subsets in lamina propria of control and examined subjects**

<table>
<thead>
<tr>
<th>%</th>
<th>Control group n = 17</th>
<th>Examined group n = 17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD4</td>
<td>CD8</td>
</tr>
<tr>
<td>χ</td>
<td>4.45</td>
<td>2.50</td>
</tr>
<tr>
<td>SD</td>
<td>2.05</td>
<td>1.80</td>
</tr>
<tr>
<td>Se</td>
<td>0.50</td>
<td>0.48</td>
</tr>
<tr>
<td>t</td>
<td>2.72</td>
<td>8.33</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 4

**Tissue distribution of T-lymphocyte subsets in epithelium and lamina propria of examined patients**

<table>
<thead>
<tr>
<th>Examined group %</th>
<th>Epithelium N = 14</th>
<th>Lamina propria N = 17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD4</td>
<td>CD8</td>
</tr>
<tr>
<td>χ</td>
<td>4.00</td>
<td>5.80</td>
</tr>
<tr>
<td>SD</td>
<td>1.95</td>
<td>3.72</td>
</tr>
<tr>
<td>Se</td>
<td>0.47</td>
<td>0.90</td>
</tr>
<tr>
<td>t</td>
<td>3.21</td>
<td>5.78</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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</table>

**Discussion**

Mechanisms of oral lichen planus that are complex and still not entirely clarified impose the need for more subtle research procedures, which might explain the complex nature of this disease. Investigations have shown that lym-
phocytes and plasma cells in the subepithelial infiltrate of oral lichen planus are an important finding [6–9]. Walsh [9] claims that cell-mediated immunity plays a major role in this disease where cytokine production of lymphocytes influences the immune response and chronicity of the disease.

Our results have shown increased T(CD3) cell values in the epithelium of examined patients versus control subjects. The frequency and distribution of T(CD3) cells in the lamina propria were significantly increased in the examined patients (25.35 ± 12.04) in comparison with the control subjects (6.95 ± 4.05). There was a statistically significant difference between them.

The predominant role of cellular immunity has also been emphasized by Takeuchi [10]. He considers humoral immunity irrelevant in the immunopathogenesis of oral lichen planus in spite of the fact that antigen-antibody complexes are a common finding in the basement membrane. Contrary to this opinion, Abell [11] proved immunoglobulin deposits that spoke in favour of activated humoral immunity.

Comparing tissue values of T(CD3) cells in the epithelium and lamina propria in our groups we found a significant increase of T-cells in the lamina propria against those in the epithelium. Our results are in agreement with those of [6–9] and are the opposite of the results of Laskaris [12].

Kilpi [7] reported an increased frequency of CD4 cells while CD8 cells were in a resting phase. This finding was contradictory to the results obtained by [13, 14] and Loning [15] who claimed that CD8 cells were the predominant type in the infiltrate. Takeuchi [10] noticed an abundant lymphocyte infiltrate of T-cells in mucous lesions, cytotoxic cells being in the central place. Kurzinger [16] thought that CD4 and CD8 cells play the major role in the T-lymphocyte strain of lichen lesions, which was in entire agreement with the statement of Takeuchi [10]. CD4 were found to be a predominant type of cell in tissue lesions in the study by Jungell [13]. CD8 cells were below normal values, but numerous organelles found in their cytoplasm verified their increased local activity.

In the studies [17, 18] cell subpopulations in lichen planus lesions were presented with CD4 and CD8 predominant cell type, although their ratio was different. The authors associated these variations with the phases of exacerbation and remission of the disease. Identical findings were reported by [8, 19]. In favour of these assumptions are the findings of Kilpi [7] who claims that, in spite of the evidently increased frequency of CD4 cells (helper/inductor), CD8 cells are responsible for the cell damage in the Malpighian layer.

Some authors like Laufer [20] think that the two large T-cell subpopulations mediate this process by direct killing whereas other authors [21, 22] think that lymphokines are involved by delayed-type hypersensitivity.

Examining the cell subsets, CD4 and CD8 cells, as well as their ratio in the lesions of oral lichen planus in our groups of examinees and controls we found an increase of CD4 and CD8 cells in the examined group as compared to the control group. CD4 in the epithelium of the examined patients showed a mild, non-significantly elevated value – 4.00 ± 1.95 versus that in the control subjects – 3.45 ± 2.05 (p < 0.4). The CD8-cell value in the epithelium of the examined group showed an almost double increase in comparison with the control group, and it reached 5.80 ± 3.72, while the CD4/CD8 ratio showed a significantly decreased value – 0.51 ± 0.12 in comparison with the control group where it was 1.38 ± 1.02 (p < 0.001). There was a highly significant difference in the CD8-cell values between the examined and the control groups, whereas there was an insignificant difference in the CD4-cell values between the examined and the control groups (p < 0.05).

Our findings demonstrated that tissue frequency and distribution of CD4 and CD8 cells in lamina propria were significantly prevalent in the examined group versus the control one (p < 0.001). An identical finding of reduced CD4/CD8 ratio as in the epithelium was found in the lamina propria, 0.68 ± 0.43 in the examined group versus 1.78 ± 1.12 in the control group (p < 0.001).

The comparison of CD4 and CD8 cell distribution in the epithelium and lamina propria in our investigation revealed an increased distribution of cell subsets in the lamina propria, resulting in a high significance. The CD4/CD8 ratio in the epithelium was 0.51 ± 0.12 as against the ratio in the lamina propria where it was 0.68 ± 0.43. These results coincide with the results obtained by [23, 24].
Distribution of T(CD3) cells, including their subpopulations, was more intensive sub-basally, that is, in the stratum papillare of the lamina propria, and one smaller segment of single or cluster-distributed T(CD3) cells along with CD4 and CD8 were located in the intra-epithelial region. Jungell [14] thinks that this finding is the cause of the disorganization of the basement membrane. He directly associates the presence and predominance of CD8 cells sub-basally with the local pathogenesis dynamics and he is still questioning whether these local tissue changes are a primary or a secondary immune response.

Increased T(CD3) cell values speak in favour of an activated cellular immune response. T(CD3) cells as well as CD8 cells have a predominant sub-basal location, but their intra-epithelial presence is not excluded. We assume that CD8 cells have a direct impact on the antigen, which most probably attacks the epithelial cells and, causing metabolic disorders, leads to changes in protein synthesis that are responsible for basement membrane creation. This impaired function of the epithelial cells is reflected in the morphological structure of the basement membrane and hence it develops fissures and irregularities, that is to say, it is disintegrated, which has been a pathohistological verified finding in patients with erosive lichen planus.

Our results have demonstrated quantitative differences in the distribution of T-lymphocyte subsets between the control and examined groups (p < 0.001). An increased distribution and frequency of CD4 and CD8 cells in the lamina propria was observed in the examined subjects in comparison with the epithelium, where the cells were predominantly located in the sub-basal region of the stratum papillare, but were also, though rarely, found in the intra-epithelial region. These findings suggest possible immune mechanisms being involved in the pathogenesis of oral lichen planus.

REFERENCES
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Резиме

Т-Клетеони Супопулацији во Лезиите на Оралниот Лишен Планус

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Цел на изучувањето: Да ја откривеме дистрибуцијата и френквенцијата на Т(CD3)-клет-