Etiopathogenetic Biochemical Mechanism Involved in Oral Lichen Planus

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Although modern science and medicine have clinical, histological, histochemical and biochemical test methods, the etiology of oral lichen planus is still relatively unknown. Recent literature supports the theory that the disease is associated with reactions that still cannot be completely defined, but began as a cellular immune response. A total of 56 patients with oral lichen planus were examined, who formed the experimental group and 50 healthy people who formed the control group. Participants of the experimental group according to clinical manifestations are divided into three subgroups: reticular, erythema - exudative and bullous form. Both groups, control and experimental, were assessed for values of T and B lymphocytes in the blood. The tests are conducted twice, in a phase of exacerbation and remission. In the phase of exacerbation in all three clinical forms of oral lichen planus, T and B cells in peripheral blood are with evident increased percentage versus the control group, resulting in high statistical significance ($p < 0.001$). In the phase of remission in reticular, erythema-exudative and bullous form has a decrease in the values of T and B cells which is still above control values and gives high significance ($p < 0.001$), except for B lymphocytes in the reticular form where $p < 0.01$. The increased values of T and B lymphocytes in the phase of exacerbation and remission can be explained by the chronicity of the process, which leads us to the conclusion that the immune system is reconstructed in terms of increased cell activity.

Keywords: oral lichen planus, reticular, lymphocytes, peripheral blood, biochemical mechanism

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Recent literature supports the theory that the disease is associated with reactions that still cannot be completely defined, but began as a cellular immune response.

Regezi et al [1] offers accurate information on the composition of the inflammatory infiltrate. The findings of the author are in favor of a general lack of plasma cells, suggesting the fact that the present cells in the infiltrates are likely T lymphocytes than B-cells.

Studies of serum lymphocytes and neutrophils in patients with oral lichen planus have proven the increase in CD4 and CD8 cell subpopulations, as well as decreasing CD4/ CD8 cell ratio [2].

Sugerman et al [3] in patients with oral lichen planus, found that the ratio of CD4 and CD45RA $^+$ T lymphocytes was significantly lower than in the control group. Furthermore, spontaneous peripheral mononuclear cell proliferation was significantly lower in patients with non reticular form of the disease. The results of the phenotypic analysis of lymphocytes from peripheral blood indicated a decreased proportion of CD3 cells and an increased proportion of T-memory cells. From the results obtained in this study it is apparent association between oral lichen planus and T-cell subpopulations. It is said that the activation of mast cells by antigen specific cellular factors initiate a whole cascade of cellular reactions. Mast cells in sensitivised tissue release vasoactive amines that act on endothelial cells, resulting in increased vascular permeability [4]

Electron microscopy supports the concept that infiltrates in the lichen planus is a cellular immune response, which is dominated by T - lymphocytes. The absence of cells with negligible presence of widened endoplasmic reticulum is a proof that it is much more cellular than humoral immune response. However in biopsy material are found much more T - cells, although there is minimal synthesis of immunoglobulins.

So far, numerous studies have provided direct evidence for the central role of the cellular immune response in oral lichen planus. Serious support of this view is the study of Schiodt [5], which clinically and experimentally proved the presence of CD4 and CD8 cells. His findings suggest that auto reactive CD4 cells may contribute to cytotoxicity, against the oral mucosa.

Despite evidence of humoral autoimmunity and cellular hypersensitivity, however, the mechanism and evolution of the disease still remain unknown, if taken into account the fact that the initial phase of oral lichen planus, according Akbar [6], is clearly associated with leukocyte movement in normal and pathologically changed oral mucosa.

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Based on previous data from the literature, we have set out the aim of this study: to follow the immune status in patients with oral lichen planus in the reticular, erythema-exudative and bullous form, in the phase of exacerbation and remission of disease, by assessing the level of T and B lymphocytes in serum.

Experimental part

To realize this goal, at the Clinic for oral pathology and periodontology - Faculty of Dentistry in Skopje, were followed 56 patients of different sex and age, diagnosed with oral lichen planus regardless of topographic distribution of the changes. These participants have formed the experimental group.

The study did not include patients neither with skin manifestations, nor those with changes on skin and oral mucosa. Diagnosis is constructed on the basis of a thorough medical history taken and objective clinical findings.

According to clinical manifestation, experimental group is divided into three sub-groups of patients; with reticular form (21 patients), erythema-exudative form (16 patients) and bullous form (19 patients). The control group was formed from 50 healthy individuals who were not registered any intercurrent disease.

At all patients with oral lichen planus, regardless of clinical manifestation, are followed changes in immune reactivity of cellular immunity by determining the levels of T and B lymphocytes in the blood.

To realize this goal, the blood is taken in the Institute for Transfusion by venepuncture from cubital vein, with anticoagulant agent in sterile test tubes, and then according to the standards procedures set for determining T and B cells.

Verification is performed by immunofluorescence when lymphocyte mass push on 2-3000 I/ in MEM. Then, in 45 microlitres lymphocytic masses are added 5 microlitres antiglobulin labeled with fluorescein (Bio Merino) and centrifuged 1 min at 500 g. The obtained precipitate is broken, stir and incubate for 30 min. on 40°C. Then washed in MEM with centrifuging 1 min. on 500 g. The supernatant is discarded. Precipitation breaks easily and make smear, which is dried and fixed 10 min. in methanol, dried again and placed one drop of glycerol in 20% PBS, covered with a shroud glass and read with a fluorescence microscope (Opton).

Anti globulin serum labeled with fluorescein binds to surface immunoglobulin of B lymphocytes in the form of points (patch) and a cap (capping). The product where erythrocytes are suspended in PBS is used to determine T lymphocytes. It is necessary to count 100 Li, of which some are free and some are associated with more than 3 Er = rosette. The number of lymphocytes that are tied in a rosette is the percentage of T lymphocytes.

The tests are conducted twice, in a phase of exacerbation and remission.

Findings were statistically processed using the Student t - test.

Results and discussions

The typical results for exacerbation phase of oral lichen planus, in patients with reticular form, and the control group are shown in table 1. Values for T (CD3) lymphocytes in control group represented 55 % and compared with values †in experimental group, which increased and amounted to 65.02 %, are in favor of a difference of values that is very highly significant ($p < 0.001$). Statistically very high elevation or significance ($p < 0.001$) existed at B - lymphocytes, where mean values †in the experimental group were dramatically increased (14.05 %), compared to the control group where they amounted to 8 % (table 1).

Table 2. shows that patients with erythema-exudative form of oral lichen planus, T (CD3) lymphocytes in the experimental group increased (69.02 ± 2.84%), compared to the control group where their value is 55 ± 9%. B-lymphocytes are also increased in the experimental group in the phase of exacerbation and amounted 17.20 ± 1.17%, versus the control which is 8 ± 2%. In both investigated parameters statistical analysis expressed very highly significant difference values ($P < 0.001$).

Findings for stage of exacerbation in patients with bullous form of oral lichen planus and control group, are shown in table 3, and they are similar. Namely, mean values of T (CD3) and B-lymphocytes at the experimental group are increased, whereas the difference in the values of the control group is very highly significant ($p < 0.001$).

The obtained results which have been achieved for the values of T (CD3) and B-lymphocytes in the peripheral blood in patients with reticular form of lichen planus, in a stage of remission and control group, are presented in table

	Control group n = 50		Examined group (phase of exacerbation) n = 21	
	T (CD3)	B ly	T (CD3)	B ly
☒	55	8	65.02	14.05
SD	9	2	4.52	0.88
Se	1.27	0.28	0.98	0.19
t			4.78	13.14
p			< 0.001	< 0.001

Table 1
VALUES OF T (CD3) AND B - LYMPHOCYTES IN PERIPHERAL BLOOD IN THE CONTROL GROUP AND IN PATIENTS WITH RETICULAR FORM OF LICHEN PLANUS IN THE PHASE OF EXACERBATION

	Control group n = 50		Examined group (phase of exacerbation) n = 16	
	T (CD3) ly	B ly	T (CD3) ly	B ly
☒	55	8	69.02	17.20
SD	9	2	2.84	1.17
Se	1.27	0.28	0.71	0.29
t			6.04	17.20
p			< 0.001	< 0.001

Table 2
VALUES OF T (CD3) AND B - LYMPHOCYTES IN PERIPHERAL BLOOD IN THE CONTROL GROUP AND IN PATIENTS WITH ERYTHEMA - EXUDATIVE FORM OF LICHEN PLANUS IN THE PHASE OF EXACERBATION

	Control group n = 50		Examined group (phase of exacerbation) n = 19	
	T (CD3) ly	B ly	T (CD3) ly	B ly
☒	55	8	67.54	16.20
SD	9	2	1.30	0.94
Se	1.27	0.28	0.29	0.21
t			5.96	16.90
p			< 0.001	< 0.001

Table 3
THE VALUES OF T (CD3) AND B - LYMPHOCYTES IN PERIPHERAL BLOOD IN THE CONTROL GROUP AND PATIENTS WITH BULLOUS FORM OF LICHEN PLANUS IN THE PHASE OF EXACERBATION

	Control group n = 50		Examined group (phase of exacerbation) n = 19	
	T (CD3) ly	B ly	T (CD3) ly	B ly
☒	55	8	58.10	11.89
SD	9	2	3.53	1.69
Se	1.27	0.28	0.77	0.30
t			1.51	7.41
p			< 0.01	< 0.001

Table 4
THE VALUES OF T (CD3) AND B - LYMPHOCYTES IN PERIPHERAL BLOOD IN THE CONTROL GROUP AND IN PATIENTS WITH RETICULAR FORM OF LICHEN PLANUS IN STAGE OF REMISSION

4. Evident from this table is growing of T (CD3) and B lymphocytes in the experimental group, with moderately expressed significance of differences in values ($P < 0.01$) for T (CD3) lymphocytes and very high significance of differences compared to the control group, in the case of B - lymphocytes ($p < 0.001$).

Similar data are encountered on tables 5 and 6 where it can be noticed that in patients with erythema -exudative and bullous form in the remission stage, there is an increase of T (CD3) and B-lymphocytes, as compared with control group and the difference between them is statistically very highly significant ($p < 0.001$).

Unclear and complex mechanisms of oral lichen planus, in modern conditions imposed need to realize what are possible subtle research procedures, which in its own way suggest the predominance of immune components in the pathogenesis of this very often disease. Finally defined attitude for predominance of humoral mechanisms are still not existed. Certainly it could be said only that there is an evident failure of a research plan, when it comes to explain the immune dynamics in patients with oral lichen planus or when necessary to interpret some of its pathogenic mechanisms.

Simon [7] and Lin [8] in their reports claim that T-cell subsets, despite greater representation found cannot be a valid parameter for the assessment of their own activity.

They point to a multicolored analysis that would render the correlation of the number of lymphocyte subsets and their function.

With the help of colored flow cytometry in patients with oral lichen planus, was found predominance of CD8 cells, while the number of CD4 was almost identical in the experimental and in the control group. CD4/CD8 ratio in patients with oral lichen planus was reduced. Overproductions of CD8 cells are associated with immunosuppression present in this group of patients [7, 8].

	Control group n = 50		Examined group (phase of remission) n = 16	
	T (CD3) ly	B ly	T (CD3) ly	B ly
☒	55	8	64.60	15.20
SD	9	2	2.40	1.21
Se	1.27	0.28	0.60	0.30
t			4.15	13.41
p			< 0.001	< 0.001

Table 5
VALUES OF T (CD3) AND B - LYMPHOCYTES IN PERIPHERAL BLOOD IN THE CONTROL GROUP AND PATIENTS WITH ERYTHEMA - EXUDATIVE FORM OF LICHEN PLANUS IN STAGE OF REMISSION

	Control group n = 50		Examined group (phase of remission) n = 19	
	T (CD3) ly	B ly	T (CD3) ly	B ly
☒	55	8	61.33	15.33
SD	9	2	1.24	1.28
Se	1.27	0.28	0.28	0.29
t			3.01	14.64
p			< 0.001	< 0.001

Table 6
VALUES OF T (CD3) AND B - LYMPHOCYTES IN PERIPHERAL BLOOD IN THE CONTROL GROUP AND PATIENTS WITH BULLOUS FORM OF LICHEN PLANUS IN A STAGE OF REMISSION

Hirota [9] observed that CD4 helper cells have positive effects on the cellular immune response, taking into account the fact that $CD4^+$ molecule consists of $CD4^+$ $Leu8^+$ + $CD4^+$ $Leu8^-$, with extremely helper properties. He concluded that $CD4^+$ $Leu8^+$ has a suppressive effect on the production of immunoglobulins by B cells. Decreased lymphocyte blast genesis simultaneously induced by $CD4^+$ $Leu8^+$ molecule can be considered as argument that supported the hypothesis of immunosuppression.

Simon [7] announces that neutrophil cell activity in oral lichen planus significantly decreases in accordance with the state of the disease. However, it was found that there is no difference between the experimental and control group neither in terms of T - cell subsets nor in terms of the activity of neutrophils [2]. In this connection, there is information that hemotaxis of polymorphs is slightly reduced, and intercellular cell killing activity is evidently reduced [7]. Active oxygen is the most important factor for induction of neutrophil function. Oxygen, mainly produced by neutrophils and macrophages, supporting killing - activity of neutrophils, which means that their response was inadequate and likely production of oxygen was much lower compared with the control group. This result indicates that despite lymphocyte imbalance, there is present lower production of neutrophils, which is also characteristic of oral lichen planus.

Quantitative analysis of CD4, CD8 cells in healthy patients with erosive and reticular form of oral lichen planus showed significantly lower values of CD4 subsets at the reticular form, while CD8 cell subsets increased. CD4/CD8 ratio was reduced, which fully coincides with the findings in erosive form [10, 11].

According to his findings, there is no clear evidence that these results indicate the possible existence of different pathogenic mechanisms in erosive and reticular form. Sugerman [12] says that oral lichen planus is a common inflammatory condition of the oral mucosa, which from the epidemiological aspect is present in 1 to 2% of the population. He includes this dermatosis in autoimmune diseases, saying that an essential element for the existence of a self tolerance suppressor function of T-lymphocytes, and impaired cell-dependent suppressor activity is a key element in the pathogenesis of autoimmune diseases.

This assumption that was proved and in vitro, provide indirect evidence for deficient suppressor cell-dependent activity in patients of oral lichen planus.

When analyzing the phenotype and function of peripheral lymphocytes in oral lichen planus, Sugerman [12] in the experimental group saw significantly declined proportion of $CD4^+$ and CD45PA + T lymphocytes, and significantly increased $CD4^+$/CD25 ratio of T ly.

The results of the phenotypic analysis of peripheral lymphocytes indicate a reduced proportion of T cells and an increased ratio of memory T cells.

According to this author, functional studies have recorded occurrence of phenotypic change, although corresponds with T-cells, which in vitro are characterized with suppressor - inductor activity. These results suggest possible link in the expression of oral lichen planus and defective T-cell internal dynamics.

Examining the production of cytokines, Kimball [13] pretty deftly interprets T - lymphocyte function. The results of his examination suggest altered immune status of patients with oral lichen planus, just in case of the enhancement of T - lymphocyte function. Analyzing serum T (CD3) cells and B-cell lymphocytes in patients with oral lichen planus in the exacerbation phase, a significant increase is evident for both groups of lymphocytes versus control group. Statistical analysis indicates a very high significant difference values ($p < 0.001$). Regarding our results, in all clinical forms, as reticular, erythema - exudative and bullous form in stage of exacerbation and remission, T (CD3) and the values of B - lymphocytes increased compared to the control group. If we follow the results of T (CD3) and B-ly in various clinical forms of oral lichen planus in the phase of exacerbation and remission, it is evident their serum levels increase over the control group ($P < 0.001$), except between the values †of reticular form and the control group, where the T ly - cells difference is moderately significant $p < 0.01$), because serum experimental group are close to those of the control group.

The present antigen stimulator causes blast transformation of T - lymphocytes, which intensively proliferate and produce offspring that initially represent large young forms - lymphoblasts, which later mature into small T (CD3) lymphocytes, which may explain the detected increased values in all clinical forms of oral lichen planus. Identically with T - cells, B - lymphocytes in our tests showed elevated values in stage of exacerbation in the reticular, erythema - exudative and bullous form; consider them to be the result of either direct or indirect antigen stimulation, whereas the signals of T - lymphocytes resulting in the expansion of B - lymphocyte clones.

Simultaneously with intensive breeding, these B cells transform into plasma cells, expose immunoglobulins on its surface, and releasing them in circulation, thus participate in the so-called humoral immune response, also in oral malignancy [14, 15].

Regarding the remission phase, at erythema-exudative form, lymphocytes are highly significant increased compared with the control group. Statistical analysis of the values of T (CD3) and B - lymphocytes in the phase of exacerbation and remission indicated highly significant difference in the values of all clinical forms, ie the apparent increase of T and B - cells in the phase of exacerbation, except in bullous form in the stage of exacerbation and remission, where statistical analysis shows low significance of differences in values ($p < 0.05$) for B-lymphocyte cells.

Kupper [16] says that in order to clarify the pathophysiology of oral lichen planus, it requires further studies on cellular immunity, directed towards detection of cell migration to the affected site and local activation of the cytokines produced by keratinocytes.

Conclusions

Serum representation of T (CD3) - cells and B - cells showed a higher index in both clinical stages. Phase of exacerbation likely due to an antigen stimulator which acts on T - cells causing blast transformation (intensive proliferation). Signals from T - cells are directly targeted at B - lymphocyte clones, which simply transform into plasma cells and start with immunoglobulin synthesis in the remission phase. The increased values of T (CD3) and B - cells are in favor of the fact that in patients with oral lichen planus even at the stage of remission, there exist some disorder of immune mechanisms, holding the body in a stage of latency, which can be easily transform into pathological.

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Manuscript received: 22.02.2015