

ROLE OF HUMORAL MECHANISMS IN ETIOLOGY OF LICHEN PLANUS

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Abstract

Aim: To examine the role of IgA, CIC and component C3 as indicators of humoral immune response in the etiopathogenesis of oral erosive lichen planus (OELP).

Material and method: The study comprised 19 patients with OELP whose samples of blood, saliva and tissue were obtained after carefully taken medical history and clinical examination. Samples of oral mucosa were taken from the site of lesion, i.e. exclusively from buccal mucosa (1 cm in width and length), and from the deep epithelium as well as a segment from the lamina propria. Determination of immunoglobulins in serum and saliva, and determination of component C3, was done using the micro-elisa technique by Rook&Cameron, Engvall and Ulman. Determination of CIC in serum and mixed saliva was done with the PEG (polyethylene glycol) method. Determination of immunoglobulin A and component C3 in biopsy material was done with direct immunofluorescence.

Results: Levels of immunoglobulin A in serum in OELP during exacerbation were decreased (1.04 ± 0.49 gr/l) and during remission increased (5.92 ± 0.62) in comparison with the control group ($p < 0.001$). Levels of CIC during exacerbation and remission were increased ($p < 0.001$), and component C3 levels were increased in both examined phases in the examined group compared with the control group ($p < 0.05$). Deposits of IgA were registered in one (5.88%) patient with OELP and component C3 was registered in 3 (17.64%) patients.

Conclusion: Changes in IgA values, as well as CIC and component C3, may correlate with changes in oral mucosa emphasizing the role of humoral immune response in the pathogenesis of oral lichen planus.

Key words: erosive lichen planus, humoral immunity, immunoglobulin A, CIC, component C3.

Introduction

The etiology of lichen planus is still not well established, but it has been confirmed that different causes participate in its pathogenesis [1, 2]. It is believed that immunologic mechanisms are involved as one of the potential factors in the pathogenesis of this common disease [163].

The immunological ground is one of the priority mechanisms in the multicausal pathogenesis of oral lichen planus in which immunoglobulins [4, 5] and autoantibodies [669] play the key roles. Literature reports related to this issue differ. Some studies have presented increased [4, 5, 8, 10, 11], reduced [11, 12] or

even normal [13615] immunoglobulin fractions in patients with oral lichen planus.

Immunoglobulins are glycoproteins, which are expressed as membrane receptors on the surface of B-cells or as soluble molecules secreting B-cells [16]. In normal serum immunoglobulin G is found in 70675% or 1000 mg/dl [17], followed by IgA accounting for 15620% or 200 mg/dl [1]. The situation is quite the opposite in saliva, the level of immunoglobulin G is reduced and reaches a maximum 263 mg/dl, and the IgA level is increased and is around 10620 mg/dl [18].

Sistig [19] says that salivary immunoglobulin levels may play a role in the pathogenesis of oral mucosal diseases, including lichen planus, or that they reflect clinical changes in these conditions. With reference to this issue, an increased level of serum IgA and IgG in patients with OLP has been found [20, 21].

Ghaliani [22] showed significant differences in the distribution of IgG + cells in different sites of oral lichen planus or lichenoid lesions separately, but there was no significant difference in the distribution of IgG + cells between oral lichen planus and lichenoid lesions. Although Sistig [19] demonstrated an increased level of salivary IgA and IgG in these patients, Van der Waal [23] did not confirm that assumption.

These facts have motivated us to set the aim of our study: to determine the role of IgA, CIC and complement C3 as indicators of humoral immune response in the etiopathogenesis of oral erosive lichen planus (OELP) by their monitoring in serum and saliva during exacerbation and remission phases as well as in tissue samples taken from the affected site.

Material and method

The study included 19 patients of different sex and age, diagnosed with oral lichen planus on buccal mucosa, who were treated according to the guidelines of the Mouth and Periodontal Diseases Clinic of the Faculty of Dentistry in Skopje and also the Ethics Committee. The study did not include patients with cutaneous manifestation and those who, in addition to cutaneous manifestation, had oral manifestation. Diagnosis was made on the basis of thorough history, objective clinical findings and pathohistological verification in the biopsy specimen.

In addition to a careful medical history and clinical examination, serum, saliva and tissue samples were taken from each patient. Blood was drawn at the Institute of Transfusion Medicine with venipuncture from the cubital vein adding an anticoagulation agent in sterile tubes.

Unstimulated 5 to 10 ccm morning saliva was collected at the Clinic of Mouth and Periodontal Diseases. Two hours after the samples were taken, the material was sent to the Institute of Transfusion Medicine, where it was frozen until analysed. Oral mucous samples were obtained by biopsy, which was performed in sterile conditions, under local anaesthesia. 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 samples were diluted in sterile PBS and distributed to the Department of Nephrology where they were frozen and then analysed.

IgA and component C3 were determined in serum, saliva and tissue samples. CIC was determined only in serum and saliva.

The examinations were done in phases of exacerbation and remission.

Determination of immunoglobulins in serum and saliva was done with the micro-elisa technique by Rook & Cameron, Engvall and Ulman.

Determination of component C3 in saliva was done with the same method as that for immunoglobulins, the difference being in the partition plates used, coated with specific antibodies for these components. Normal serum values for C3 are 0.8061.40 gr/l, and for C4 0.26 0.5 gr/l.

Determination of CIC in serum and mixed saliva was done with the PEG (polyethylene glycol) method.

Determination of IgA and component C3 in biopsy specimens was done with direct immunofluorescence.

The tissue was frozen at -28° in cryocut, and then cut. Samples were dried for 30 minutes at room temperature. Thirty minutes later they were put in cuvettes with a cold acetone where they were kept for 10 minutes; when rabbit serum is prepared in acetone diluted with PBS (NaCl 29.2 gr + $\text{NaHPO}_4 \cdot \text{H}_2\text{O}$ 2.5 gr +

$\text{NaHPO}_4 \cdot 7\text{H}_2\text{O}$ – 14.5 ratio for the solution of 4l) in 1: 5 ratio. 100 μl serum and 500 ml PBS are taken and kept in tubes. Following this procedure, samples are placed in a humid chamber, a drop of rabbit antiserum is poured in; the tissue is covered and is left in a humid chamber for 10 minutes. While samples are in the chamber, sera are diluted.

For determination of IgA 5 μl serum and 250 ml PBS are taken. Ten minutes later the excess rabbit serum is removed from the samples, but they have to be kept humid; then, a drop of the relevant antiserum with monoclonal antibodies is added to each sample; the tissue is covered and left in a humid chamber for 90 minutes.

Anti-mouse peroxidase conjugate (prepared serum) in a volume of 20 μl is added and diluted with 800 ml PBS. 40 μl of human serum is added to the diluted serum. Serum is prepared in the phase of washing the samples. Samples are washed with PBS in cuvettes twice in two minutes. They are dried, carefully mopped and put in a humid chamber.

A drop of the prepared anti-mouse peroxidase conjugate is poured on each sample so that they are well covered and are left in a humid chamber for 30 minutes. Then, they are washed with PBS three times for 3 minutes. In the meantime, a 5 mgr solution of diaminobenzidine is prepared in a tube with 10 ml PBS. The test-tube is well mixed and in another tube 3 drops of 3% H_2O_2 are added. These two mixtures are combined and mixed. A drop of this mixture is poured on the samples and they are left in a humid chamber for 1 to 2 minutes. Diaminobenzidine is removed from the samples; they are again placed in PBS and are washed; then they are put in hematoxylin for 0.5 to 2 minutes; they are washed with water several times for 2 to 3 minutes. Then they are kept in 96% alcohol three times for 2 to 3

minutes, then they are put in xylol and finally they are filtered.

One drop of canada balsam is poured onto (cover) glass slide and attached to the slide. The sample is wrung well and carefully examined under a microscope.

The results obtained were compared with those of the control group as well as among the patients during exacerbation and remission periods.

The control group consisted of 224 healthy subjects from the Institute of Transfusion Medicine in Skopje who did not suffer from lichen planus or any other intercurrent illness.

The results of salivary CIC and IgA levels were used for comparison with a control group of 25 subjects who did not suffer from lichen planus or any other intercurrent illness.

The results obtained for the levels of C3 in serum in the control group from the Institute of Transfusion Medicine were used for comparison.

All results were compared with the control group and among the patients in the phases of exacerbation and remission.

The results were statistically analysed by using the Student's t-test for significance in differences.

Results

Statistical analyses of the values of IgA in serum in OELP during exacerbation showed decreased levels (1.04 ± 0.62) and during remission increased levels (5.92 ± 0.62) in comparison with the control group, resulting in both cases in highly significant differences ($p < 0.001$). CIC values in exacerbation and remission were increased ($p < 0.001$). C3 values were slightly increased in both examined phases in our groups of examinees and controls ($p < 0.05$) (Table 1).

Table 1

Serum levels of IgA, CIC and component C3 in examined and control groups during exacerbation and remission

gr/l	Control group			Examined group					
				Exacerbation			Remission		
	IgA	CIC	C3	IgA	CIC	C3	IgA	CIC	C3
	2.70	0.05	0.65	1.04	0.14	0.90	5.92	0.11	0.75
SD	0.63	0.02	0.14	0.49	0.09	0.13	0.62	0.04	0.17
Se	0.04	0.001	0.02	0.11	0.02	0.02	0.14	0.009	0.03
t				11.15	11.84	2.73	21.33	11.25	2.08
p <				0.001	0.001	0.05	0.001	0.001	0.05

Salivary IgA levels in oral erosive lichen planus during exacerbation and remission phases varied identically as in serum ($p < 0.001$). CIC values during exacerbation and remission were increased compared to the control group ($p < 0.001$). CIC values showed an increase in

both phases in the examined compared with the control group ($p < 0.001$), and C3 showed a more dramatic decline during exacerbation ($p < 0.001$) and moderate decline during remission ($p < 0.05$) (Table 2).

Table 2

Salivary IgA, CIC and component C3 levels in examined and control groups during exacerbation and remission

Control group				Examined group					
				Exacerbation			Remission		
gr/l	IgA	CIC	C3	IgA	CIC	C3	IgA	CIC	C3
	1.10	0.02	0.76	0.71	0.09	0.28	2.42	0.04	0.57
SD	0.01	0.01	0.23	0.31	0.03	0.09	0.09	0.02	0.19
Se	0.002	0.002	0.004	0.007	0.006	0.02	0.02	0.004	0.04
t				6.14	10.64	8.41	13.20	4.23	2.35
p <				0.001	0.001	0.001	0.001	0.001	0.05

Table 3 presents tissue distribution of IgA and component C3 in both controls and examinees. At the basement membrane no deposits of IgA and component C3 were found in

the control group; IgA deposits were found in one (5.88%) of the examined subjects, and complement C3 was found in 3 (17.65%) patients (Figs. 1 and 2).

Table 3

Tissue distribution of IgA and component C3 in control group and bullous oral lichen planus

	Control group n = 14		Examined group n = 17	
	n	%	n	%
IgA	0	0	1	5.88
C3	0	0	3	17.64



Figure 1 – Deposits of IgA at the epithelial basement membrane and the most superficial layer of lamina propria in OELP



Figure 2 – Deposits of component C3 at the basement membrane, suprabasement in tissue specimens of OELP

Discussion

Immunoglobulins are carriers of humoral immunity, and hence every increased or reduced value of certain groups of proteins is an indicator not only of the existence of some morbid process, but also of the degree of its activity, no matter what its location and specificity.

Regarding this, Sklavounou [24] pointed out a significant reduction of all immunoglobulin fractions, including IgA in serum. Examining a quite small number of cases, Stankler [25] concluded that there was a significant lowering in the levels of both IgA and IgM. Contrary to these findings, Biocina-Lukenda [26] documented increased serum levels of IgA and IgM in oral lichen planus, suggesting that humoral immunity participated in the pathogenesis of oral lichen planus.

Jacyk and Greenwood [27], in an attempt to test the findings of Stankler [25], observed a significant decrease only in the IgM level, whereas they found no quantitative deviation in the remaining immunoglobulin fractions.

Cerni and Griffith [28–29] reported normal levels of all immunoglobulins, including IgA, as well as of component C3.

Our findings have revealed a significant decrease of IgA in serum during exacerbation in comparison with the control group ($p < 0.001$). This result is partially in agreement with the findings of some authors [2, 5, 25, 27], and the opposite of the findings of other authors [29]. With reference to the values of this immunoglobulin in the phase of remission, we observed an increase in the examinees compared to the controls ($p > 0.001$).

Sallay [30] emphasizes that a subepithelial mononuclear reaction is a special characteristic of lichen, and that it is aimed at the epithelial cells leading to destructive zones in the affected surfaces. Identified deposits of immunoglobulin, complement, fibrinogen and CIC in the lichen planus lesions enter through circulation [31]. Chatterjee [32] observed markedly higher CIC mean values in several complicated oral hyperkeratosis, such as leukoplakia, epithelial dysplasia and oral carcinoma. This author claims that CIC in the tissue originates from the circulation and is a result of some pathologic conditions in the organism. The hypo-

thesis of Chatterjee [32] is in contrast to the findings of Weksler [33].

Association of free humoral antibodies in CIC might be induced by various diseases, especially diabetes mellitus or hypertension, that is, the so-called Grinspan's syndrome. Thus it is logical that high serum levels of CIC are present in this group of patients [32].

This conclusion coincides with Weksler's observation [33]; he states that any deviation in terms of elevated or reduced CIC serum concentration is not a reflection of some morbid process in the organism, but rather of physiological variations that go parallel with ageing. Sallay [30] found an increased frequency of CIC positivity in str. granulosum and str. spinosum in biopsy specimens of lichen lesions.

Within this context Banoczy [34] observed an increased frequency of CIC positivity not only in oral lichen planus, but in oral leukoplakia and oral carcinoma as well. According to this author, this parameter points to the possibility that CIC positivity, both in serum and in tissue, in patients with oral lichen planus and leukoplakia, is an indicator of an eventual malignant transformation of these diseases.

In our examined group the CIC level in serum during exacerbation and remission was increased in comparison with the level in the control group ($p < 0.001$).

Our results obtained for the CIC levels are identical with those reported by some other studies [30, 32, 34] and are in contrast with the findings of Weksler [33].

The complement is one of the major humoral effector mechanisms of particular importance. Tissue disorders are usually destructive changes caused by immune complexes. Deficiency or hyperproduction of some of the components might result in the development of some disease [35]. Not observing any changes in the component C3 level, the theory about its role in the pathogenesis of oral lichen planus was completely rejected [24, 29, 36].

Our findings obtained for component C3 have shown moderately increased values in exacerbation, and thus there was a small statistical significance of difference in the values ($p < 0.05$). In the remission phase the level of component C3 was decreased as opposed to the exacerbation phase (0.75 ± 0.17 gr/l). As we have already mentioned, in our examined subjects in

the phase of disease exacerbation there was a reduced serum level of IgA and an increased level of CIC. We think that these low levels of serum IgA and concomitant increase of CIC are due to their incorporation into the CIC. CIC, which is associated with IgA, which activates the complement in an alternative way. The high CIC level in this phase of the disease indicates an activated humoral response by including the complementary system as a major mediator in the antigen-antibody reactions. We can explain the obtained high levels of component C3 in this phase of the disease with the emphasised and indisputable role of the complementary system in the etiopathogenesis of oral lichen planus.

The results of our examination in the remission phase showed an increased serum IgA. CIC was also increased and component C3 was moderately decreased in comparison with the phase of exacerbation, but still higher than the results in the control group.

It is known that oral lichen planus is a disease that persists throughout the lifespan in the majority of cases, with occasional exacerbations or spontaneous and therapeutic improvement, and with shorter or longer remission periods. Therefore, the opinion of many oral pathologists is justified concerning the need for a certain antigen, of unknown origin at present, which would stimulate or inhibit the immunologic response of the organism.

In the phase of remission in patients with the erosive form there is epithelization of the previously present erosive or erosive-ulcerous surfaces, with or without minimum signs of inflammation and improved general subjective symptoms. Stabilization of the immune status in this phase of the disease was manifested by an increase of the immunoglobulin fraction in our group of patients. The factor (antigen) that stimulates the immune system is most probably present somewhere in the organism, but provocation that will activate it is necessary and will cause the phase of exacerbation. The present immunoglobulins in the circulation during re-entry of the antigen will join the antigens and will create CIC by incorporating the component C3. This, in fact, explains the high levels of CIC and reduced levels of component C3 in the remission phase as opposed to the exacerbation phase.

One of the numerous functions of the complement is cell lysis, which is being realized through complement components C8 and C9. This action is performed by the complement which binds the component C3 and later all other components, which is reflected by a decreased level of component C3 in the phase of remission.

The results obtained in our group of patients in the phase of disease exacerbation have shown reduced salivary IgA levels (0.71 ± 0.31 gr/l) as opposed to the results in the control group (1.10 ± 0.01 gr/l), and hence the difference between them was statistically significant ($p < 0.001$). It has been proven experimentally and theoretically that IgA is the major protein with a protective function, which is in direct relation with the defence mechanism of the oral mucosa. We can explain the reduced levels of this immunoglobulin with the reduced resistance of oral mucosa to numerous exogenous and endogenous factors that attack it frequently and induce certain pathological conditions manifested with possible classic, benign and even erosive-ulcerous lesions, with a very dramatic clinical picture and even possible malignant alteration. In the phase of exacerbation this immunoglobulin is created to a certain extent from the serum or as a result of its local synthesis in the salivary glands. However, the ability of this immunoglobulin to activate the complement as well as to bind with the antigen-stimulator, to incorporate in CIC with the saliva, leads to reduced salivary levels of the C3 that is incorporated into this complex. This is how we interpret our results that are characteristic for this phase of the disease. If we add the ability of this immunoglobulin to increase the phagocytic activity of macrophages and to have an indirect bacteriolytic effect, which is realized in the presence of the complement and lysozyme, then the low levels of component C3 obtained in our investigation were to be expected. One part of IgA from the mixed saliva is bound to the mucin-creating precipitating cover that is "the first line of defense", which in a certain way incorporates this immunoglobulin and hence accomplishes one of its basic functions – a bioprotective one. This is also an explanation of the results we have obtained.

We observed an increase of IgA in the phase of remission in patients with OELP. The

difference between the examined and control groups was highly significant ($p < 0.001$). We can explain the increased salivary IgA levels in the remission phase with strengthening the general immunological status of the organism, which has some effect on the local defence capability of the oral mucosa and which is reflected in the objective and subjective improvement of the clinical presentation of the disease in all clinical forms of oral lichen planus.

Sistig et al. [37] did not define difference between exacerbation and remission, but they found increased salivary levels of IgA in oral lichen planus.

It is known that IgA is the key antibody in all secretions, including saliva, and it is completely responsible for realization of the defence mechanisms of mucous surfaces. Although in this context the priority is given to this immunoglobulin, the other carriers of humoral immunity are not to be neglected.

The analysis of CIC in exacerbation and remission revealed an increased level in comparison with the control group, and a decreased level of component C3 in the examined group.

Numerous former morphological studies have provided only limited information on the nature of this disease. In the beginning the changes in the epithelium were pointed out as crucial and responsible for the pathogenic events, with a special accent on the degenerative changes of the basal cells [38–39].

Walker [41] examined the immunological markers in the lesions of mucous membrane in patients with lichen planus and identified only a few cells in the infiltrate that belonged to B-cells or macrophages. He proved that T-cell subpopulations were predominant in the lichen planus infiltrate.

Identical results were presented by Abell [41] and Tuffanelli [42]. They consider humoral immune system or B-cells to play a smaller role in the development of this disease. Rarely found B-cells in the infiltrate are a confirmation of their results.

The concept that humoral immunity is not of crucial importance in the pathogenic sense has been supported by the latest investigations and knowledge that speak in favour of the fact that B-cells are found in infiltrates and these are precursors of cell creating antibodies. However, Yanossy [43] identified only a minimum synthesis of the immunoglobulins in the biopsy specimens compared to other diseases

but, on the other hand, lymphocytes and histiocytes were predominant.

Applying the immunofluorescent technique for detection of deposits of IgG, IgM, IgA and component C3, and fibrinogen at the basement membrane in lichen planus lesions, Schiodt [31] confirmed that tissue distribution of immunoglobulins is not typical for lichen planus lesions, although this author adds a possible diagnostic or differential diagnostic importance to these deposits. Component C3 frequently appears in other oral lesions, diametrically different from oral lichen planus, and according to Schiodt it is not pathognomic for this disease.

Frequency, distribution and morphology of the deposits of immunoglobulins, component C3 and fibrinogen in oral lesions and clinically normal oral mucosa were in accordance with the skin findings in the Konrad's investigation [44]. The findings coincided with previous examinations of the skin and oral mucosa. It is believed that not only the presence of deposits of immunoglobulins is important, but also frequency of immunoglobulin deposits at the basement membrane as well. A significant difference was discovered between discoid lesions on one side and oral lichen planus on the other [31].

Laskaris [46] observed deposition of IgA, IgG and IgM, but there were no cases with component C3. In spite of the presence of these proteins, the author thinks these findings have no diagnostic significance although sometimes they might be useful in the diagnostics of the disease.

Daniels [46] retrospectively analysed direct immunofluorescence findings in several dermatoses including lichen planus, and he noticed no positive fluorescent patterns with anti-Ig or anti-C3 complement in any samples. Contrary to this author, some other authors [47–50] discovered deposits of IgA, IgG and IgM in oral lichen planus.

In the biopsy material of our examined subjects comprising patients with erosive form of lichen planus, deposits of immunoglobulins at the basement membrane were found. Deposits of immunoglobulin A were found in one of the 17 subjects or in 5.88%. Component C3 was found in 3 patients or in 17.64%.

Our results coincide with other reports [47, 48, 50] and are contrary to some others

[38, 39, 41, 42]. Many of the local inflammatory cells in the infiltrate of the oral lichen planus lesions were the immunocompetent T-lymphocytes, which findings are in line with other authors [51–54]. Therefore, a hypothesis has been proposed about the interaction between cellular and molecular signals in the local immune response. It is based on mast cell degranulation and changes in the blood vessels endothelium, which support the lymphocytic attachment to the tissue. Cytokine production by the lymphocytes has a direct influence on local immune response, but also on disease chronicity [51].

Changes in the IgA levels on all examined media, as well as of CIC and component C3, may correlate with the changes of oral mucosa emphasizing the role of humoral immune response in the pathogenesis of oral lichen planus.

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- LICHEN PLANUS**
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19

"t"-

(1,04 \pm 0,49 gr/l),
(5,92 \pm 0,62)
(p < 0,001).

5 10 ccm.

(p < 0,001), 3

(1 cm

), p < 0,05.

PBS, p < 0,001.

(p < 0,001), a 3 e
(p < 0,001)

Rook & Cameron, Engvall (p < 0,05)

and Ulman.

3-

... 5,88%, 3-

17 ...

64%.

PEG ().

3

3

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