J Oral Pathol Med (2004) 33: 386–90

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Elevated serum levels of the apoptosis related molecules TNF-α, Fas/Apo-1 and Bcl-2 in oral lichen planus

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BACKGROUND: The serum circulatory levels of apoptosis related molecules measured in patients with oral lichen planus (OLP) and healthy individuals in order to investigate possible alterations associated with the clinical forms of OLP.
METHODS: Serum levels of tumor necrosis factor (TNF)-α, soluble Fas (sFas) and Bcl-2 studied by enzyme-linked immunosorbent assay in whole blood samples in 13 OLP reticular, 13 OLP atrophic-erosive form patients and 26 healthy subjects.
RESULTS: Significantly elevated levels of TNF-α and sFas detected in OLP patients as compared with controls. Serum concentrations of Bcl-2 although increased in 17/26 patients, they were not statistically significant. Reticular OLP exhibited slightly elevated TNF-α and significantly elevated Bcl-2 serum levels, compared with erosive OLP.
CONCLUSIONS: These data suggest that a putative dysfunction in the Fas/FasL mediated apoptosis might be involved in the OLP pathogenesis. A downregulation of Bcl-2 serum levels in the atrophic-erosive OLP may be associated with promotion of the disease activity.
J Oral Pathol Med (2004) **33**: 386–90

Keywords: apoptosis; Bcl-2; Fas/Apo-1; lichen planus; mouth; serum levels; tumor necrosis factor-a

Introduction

Apoptosis plays an important role in the pathogenesis of a wide variety of diseases including autoimmune disorders, neurodegenerative diseases and cancer. This process is genetically regulated involving several groups of molecules such as the *Bcl-2* gene family, the tumor necrosis factor (TNF) receptor/ligand families, tumor suppressor genes or oncogenes (1). The *bcl-2* proto-oncogene encodes a 26-kDa membrane protein (Bcl-2), which is essential for the maintenance of the immune system through the inhibition of apoptosis (1). TNF-α, a cytokine generated from macrophages and activated T-lymphocytes, plays either a protective or a pathologic role in inflammation and immunological reactions depending on the magnitude of the inflammatory reaction and the amount of TNF-α production (2).

Fas (Apo-1 or CD95) is a cell surface protein member of the TNF/nerve growth factor receptor superfamily and is expressed on a variety of both lymphoid and non-lymphoid cells. The ligand of Fas (FasL) belongs to the TNF family and is widely expressed in adult tissues in particular neutrophils and activated lymphocytes, in immune privileged tissues (1, 3, 4). The Fas/FasL system induces apoptosis and dysfunction in this apoptotic pathway may be involved in the pathogenesis of various autoimmune disorders. Both Fas and Fas-L have membrane-bound and soluble forms (5, 6). The soluble form of Fas (sFas) can inhibit T lymphocyte-mediated cytotoxicity *in vitro* and alter lymphocyte development and proliferation in response to self-antigen *in vivo*. It has been postulated that sFas release may protect cells from Fas-mediated apoptosis by inhibiting Fas/FasL interactions (6).

Oral lichen planus (OLP) is a relatively common chronic inflammatory disease, which can be classified into two main clinical types, the reticular (RetLP) and the atrophic-erosive (ErLP) form (7). The disease is lymphocyte-mediated and local release of cytokines is thought to interfere with lymphocytes as well as promotion of apoptotic death of basal keratinocytes (8–10).

Previous studies regarding the apoptosis-related molecules in OLP have been conducted *in situ* (11–15). The proposed apoptotic mechanisms within the OLP lesional epithelium include the TNF-α/TNFα receptors system mediated by CD8+ cytotoxic T cells, the Fas/FasL-mediated apoptosis, or through the perforin-granzyme B pathway (16). The rate of apoptosis appears to be increased in OLP epithelium compared with normal controls (12, 13, 15). However, a significantly increased cell proliferation, but not apoptosis, has been observed in the ErLP compared with the RetLP epithelium suggesting possible differences between the two clinical

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Accepted for publication December 23, 2003

forms in the process of terminal keratinocyte differentiation or in the pathway of apoptotic cell death (17). The fact that differences between the clinical forms of OLP have also been detected in the peripheral T-cell subsets suggests that an alteration to cellular immunity associated with the disease may not be restricted to the oral mucosa (18, 19).

In other previous studies (20, 21) and our own (22) elevated serum levels of TNF-α were detected in OLP patients as compared with controls suggesting a regulatory role for this cytokine in the initiation and progression of the disease. The serum circulatory levels of Fas/Apo1 and Bcl-2 have been studied in many disorders including diseases of autoimmune etiology (23, 24), but they have, to our knowledge, not been investigated in the OLP. It was the purpose of the present study to measure the serum circulatory levels of TNF-α, sFas and Bcl-2 in patients with OLP and healthy individuals in order to investigate possible alterations associated with the clinical forms of OLP and gain more insight into the pathogenesis of the disease.

Material and methods

Serum samples were obtained from 26 patients, 18 females and eight males with mean age 47.5-years old, who presented to the Department of Oral Pathology and Surgery, School of Dentistry, University of Athens. There were 13 OLP patients with the reticular form and 13 patients with the atrophic-erosive form of the disease. Diagnosis was based in all cases on clinical and histopathologic criteria (25). Clinically, 20 of the 26 patients showed bilateral buccal lesions. In addition to the buccal lesions, nine patients had tongue lesions, six had gingival lesions, three had lip lesions and one had lesions in the floor of the mouth. There was no history of receiving medication and none of the OLP patients had been treated with topical or systemically steroids. Control serum samples were obtained from 26 healthy donors, 15 females and 11 males with mean age 37.8 years. Informed consent was obtained from all patients and healthy controls. The Ethical Committee of the School of Dentistry, University of Athens, approved the protocol.

Differences in soluble TNF-a, sFas, and Bcl-2 between OLP patients and healthy donors were determined by means of the enzyme-linked immunosorbent assay. All blood samples were centrifuged and collected sera were stored at −20°C for up to 1 week. Determination of serum levels (U/ml) of TNF-a, sFas, and Bcl-2 was performed, according to the assay's protocol (PREDICTA; Genzyme Diagnostics, Cambridge, MA, USA).

Comparisons between groups were statistically analyzed using Student's *t*-test and Pearson test with continuity correction. Two sided tests were used to calculate probability values (*P*). Statistical significance was set at *P*-values <0.05.

Results

The serum levels of the apoptosis-related molecules TNF-α, sFas and Bcl-2 in the OLP patients and normal controls are shown in Table 1. Significantly elevated levels of TNF-α and sFas were detected in the sera of OLP patients as compared with controls ($P < 0.05$) as shown in Figs 1 and 2. Serum concentrations of Bcl-2 although increased in 17 of 26 patients, they were not statistically significant (Fig. 3).

In relation to the clinical form of the disease, reticular form exhibited significantly elevated Bcl-2 serum levels compared with the erosive form of OLP (Table 2; Fig. 4). Eight of 13 patients with the erosive OLP form displayed decreased serum levels of Bcl-2 compared with healthy controls, but the difference was not statistically significant. The serum TNF-α and sFas levels did not significantly differ between the two clinical groups.

Table 1 Serum levels of TNF-α, sFas and Bcl-2 in lichen planus patients and normal controls

	Lichen planus (n = 26)	*Controls (n = 26)*
TNF-α[a]	5.2 ± 3.1 (2.1–18.1)	3.1 ± 1.2 (1.2–7.2)
sFas[a]	7.5 ± 2.8 (2.1–12.8)	5.3 ± 2.1 (2.3–11.2)
Bcl-2	198.1 ± 74.1 (79–380)	175.4 ± 129.6 (56–542)

Values are given as mean value ± SD (range).
[a]$P < 0.05$.

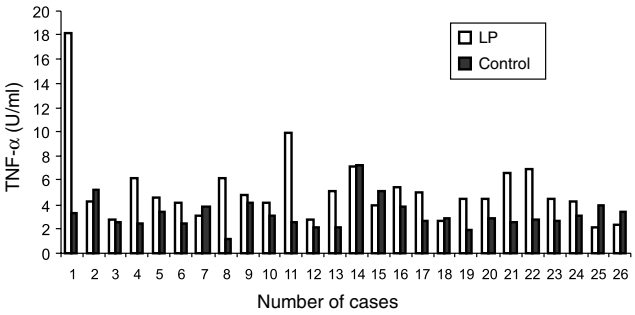

Figure 1 Serum levels of TNF-α in LP patients and normal controls.

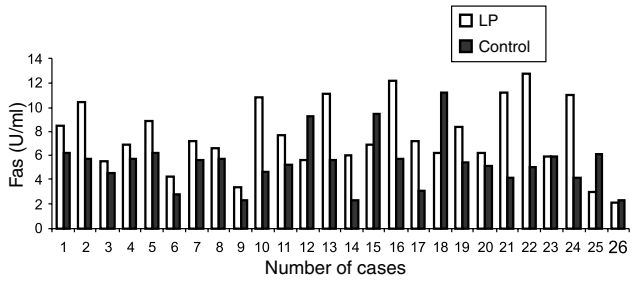

Figure 2 Serum levels of sFas in LP patients and normal controls.

Discussion

The initial event in OLP lesion formation and the factors that determine OLP susceptibility remains incompletely understood (10). However, several studies have indicated a defective peripheral immune suppressor function suggesting a role of autoimmunity in the

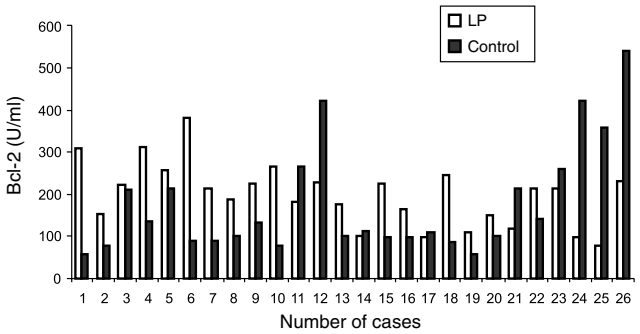

Figure 3 Serum levels of Bcl-2 in LP patients and normal controls.

Table 2 Serum levels of TNF-α, sFas and Bcl-2 in the two clinical forms of lichen planus (LP)

	Reticular LP (n = 13)	*Atrophic-erosive LP (n = 13)*
TNF-α	5.83 ± 4.13	4.56 ± 1.64
sFas	7.44 ± 2.43	7.63 ± 2.1
Bcl-2[a]	238.84 ± 64.50	157.46 ± 60.89

Values are given as mean value ± SD.
[a]$P < 0.05$.

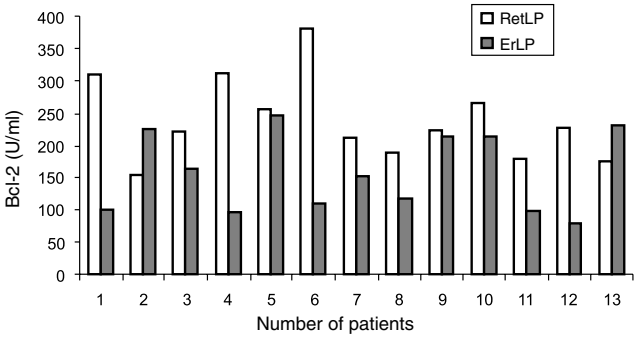

Figure 4 Serum levels of Bcl-2 in reticular LP(RetLP) and erosive LP(ErLP).

pathogenesis of the disease (8, 10, 26). Both pro-inflammatory (TNF-α and IFN-γ) and immunosuppressive (TGF-β1) cytokines are present in OLP the relative activity of which may determine the level of immunological activity in OLP lesions and the clinical behavior of the disease (16). It is believed that the atrophic-erosive form of OLP constitutes an active disease phase based on the clinical appearance of the lesions and the pathological features of the ErLP epithelium which exhibits reduced epithelial thickness and increased epithelial cell proliferation (17).

In the present study, TNF-α serum circulatory levels were significantly increased in OLP patients compared to normal controls, which is in agreement with earlier investigations (20–22) and reinforces the view that TNF-α is involved in the pathogenesis of the disease. The elevated TNF-α serum levels may be associated with the induction and/or perpetuation of the pathogenetic and apoptotic events in the lesional OLP epithelium (21). Apoptosis of the TNF-α receptor 1 expressing basal keratinocytes is possibly triggered by T-cell secreted TNF-α through the TNF-α ligand/receptor pathway, although other mechanisms of cell death can not be excluded (16).

It is not clear whether a possible fluctuation in the TNF-α serum levels may be related to the activity of the disease. In our series, TNF-α serum levels were slightly elevated in patients with RetLP compared with those with ErLP, but the difference between the two clinical forms of OLP was not statistically significant. In the study of Yamamoto et al. (20), TNF-α serum levels in patients with OLP remained elevated after the clinical improvement of the OLP lesions. The authors (20) suggested that continuous stimulation for TNF-α production from infiltrating mononuclear cells and peripheral lymphocytes remains even during the remission phase of the disease.

Fas-mediated apoptosis plays a vital role in the immune system. Dysfunction in this apoptosis system is most likely to be involved in the initiation and/or exacerbation of various immunological disorders (3–5). Among its putative mechanisms sFas, which is a soluble splice variant of Fas, would function as an inhibitor of Fas/FasL interactions (6). Elevated serum levels of sFas may block apoptosis of autoreactive lymphocytes, which could be important in the pathogenesis of autoimmune diseases. Significantly increased serum circulatory levels of sFas compared with healthy controls have been found in various diseases such as silicosis (27) and non-hemopoietic malignancies (28), as well as in conditions of autoimmune etiology such as systemic lupus erythematosus (SLE) (23) and primary Sjögren's syndrome (29).

The sFas serum levels in patients with active stages of Behcet's disease are increased compared with those in inactive Behcet's disease, SLE and rheumatoid arthritis (30). Patients with multiple sclerosis in the active phase had significantly higher Fas serum levels than those in the inactive phase of the disease or in controls (31). It has been postulated (23), that sFas serum levels could serve as an appropriate marker for evaluating SLE disease activity, because of the correlation found between elevated serum levels of sFas, laboratory findings and clinical picture in patients with SLE. The elevated levels of TNF-α in patients with SLE have been found to be positively correlated with the sFas levels (32). Miret et al. 2001 (32) suggested that in SLE an overproduction of TNF-α, which is enhanced in inflammatory conditions, may stimulate the secretion of sFas leading to inhibition of apoptosis of the autoreactive lymphocytes.

The results of the present study showed that serum levels of sFas were significantly increased in patients with OLP compared with healthy subjects, but an association with the clinical form of the disease was not observed. This finding suggests that a putative dysfunction in the Fas/FasL mediated apoptosis might be involved in the pathogenesis of OLP. In a recent study (33), anti-keratinocyte auto-cytotoxic T-cell clones have been identified in OLP suggesting a role for

autoimmunity in the disease. Elimination of putative autoreactive lymphocytes through Fas/FasL mediated apoptosis may be blocked by an upregulation of sFas, which may antagonize the Fas membrane-bound form in OLP lesions. This hypothesis can be supported by the low rate of apoptosis observed in the subepithelial T-cell infiltrate, despite the prominent FasR/FasL expression throughout the inflammatory cells in OLP (15). The sFas release may be one possible mechanism involved in T cells escape from apoptosis leading to their accumulation locally in OLP.

It has been postulated that the disease activity in OLP may be determined by the balance between keratinocyte apoptosis triggered by infiltrating T cells vs. T-cell apoptosis triggered by keratinocyte-derived TNF-α (16). Another molecule that may account for the T cells survival acting as an inhibitor of apoptosis in OLP is the Bcl-2 protein. Elevated levels of Bcl-2 have been detected in patients with active SLE compared with inactive SLE suggesting that self-reactive lymphocytes related to autoimmune phenomena, may avoid apoptosis by the overexpression of Bcl-2 (24). The Bcl-2 overexpression produces an enhancement of cell survival and seems to have a role in maintaining the lymphocyte hyperactivity and the active state of the SLE (32). The Bcl-2 serum levels have been found to be elevated in patients with multiple sclerosis in the active phase than those in patients in the inactive phase or in controls (31). In contrast to autoimmune diseases, the Bcl-2 serum levels are decreased in HIV-1 infection suggesting that downregulation of this apoptosis-inhibitory protein may play a role in the T-cell elimination by apoptosis (34).

In our study, there were no significant differences in serum Bcl-2 levels between all OLP patients and healthy controls, although elevated Bcl-2 levels were detected in 17 of 26 patients. Interestingly, the serum Bcl-2 levels in ErLP were significantly decreased compared with the RetLP patients. This finding suggests that a possible downregulation of the Bcl-2 protein in the atrophic-erosive form of OLP may be associated with T cells apoptosis susceptibility in the subepithelial infiltrate. The Bcl-2 protein does not seem to be involved in the epithelial changes in OLP, because the keratinocytes show no immunoreactivity for this protein (11, 14). An increased rate of apoptosis has been observed in the intra- and subepithelial inflammatory infiltrate in areas with atrophic LP epithelium (15). Sugerman et al. (35) identified mixed helper and suppressor activity among OLP lesional T-cells clones *in vitro*. Inadequate immunosuppression may promote hyperactive immune responses in OLP and a possible selective apoptosis of putative immunosuppressive T cells in OLP may be associated with promotion of the disease activity. However, it is difficult to contemplate why cytotoxic T-cells may escape apoptosis maintaining a possible hyperactivity of the disease. Further studies are required to investigate the biologic importance and clinical significance of Bcl-2 serum levels alteration between the OLP clinical forms, and in comparison with other oral mucosa diseases of autoimmune pathogenesis.

Conclusions

The detection of increased TNF-α serum levels in OLP patients vs. normal controls, reinforce the concept that this molecule may play a key regulatory role in the initiation and progression of OLP, as well as the induction of apoptotic events in the lesional OLP epithelium. The increased levels of sFas in patients with OLP suggest a putative dysfunction in the Fas/FasL interactions, which may be involved in T cells escape from apoptosis. Finally, there was no difference detected in TNF-α and sFas serum levels examined between reticular and atrophic-erosive OLP indicating that there might be a large degree of overlap in the pathogenesis of the various OLP clinical forms. The difference between the two clinical forms in the Bcl-2 serum levels suggests that downregulation of this anti-apoptotic molecule in the atrophic-erosive OLP may be associated with promotion of the disease activity.

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Acknowledgements

This study was supported by Special Account for Research Grants of the National and Kapodistrian University of Athens (code 70/4/3269).