Oral lichen planus: immunohistology of mucosal lesions

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Abstract
Background: Current evidence suggests that immunological mechanisms are involved in oral lichen planus (OLP) pathogenesis. The events implicate activated epithelia that comprise antigen-presenting Langerhans cells, immunocompetent keratinocytes and subepithelial inflammatory infiltrate. Also, the presence of a high density of leucocyte cells may occur for the expression of a variety of adhesion molecules. The aim of this study was to analyse the immunoexpression of some adhesion molecules as well as lymphocytic markers in order to determine the disease pathogenesis in a Venezuelan population.
Methods: The 18 OLP and 10 normal oral mucosa biopsies were immunostained for CD4, CD8, CD1a, LFA-1, VCAM-1 and ICAM-1.
Results: The results showed an increased number of CD4+, CD8+, CD1a+ cells in OLP. Serial sections showed CD4+ and CD8+ cells also expressed LFA-1. The expression of ICAM-1 and VCAM-1 were significantly higher in OLP.
Conclusions: The immunological reaction begins with Langerhans cells activation, which presents an antigen to CD4+ lymphocytes. Those cells through ICAM-1 and LFA-1 promote epithelial destruction. Afterwards, cytokine production, ICAM-1 and VCAM-1 expression can activate CD8+ lymphocytes leading to the chronic form of the disease.

Key words: adhesion molecules; dendritic cells; oral lichen planus; oral mucosa; T-lymphocytes

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Oral lichen planus (OLP) is a chronic disease, which can present as white patches or erosions of the oral membrane (1). Although the cause of LP remains unknown, current evidence suggests that immunological mechanisms are involved in its pathogenesis (2, 3).

The histopathological diagnosis is based on the presence of an infiltrate of mononuclear cells in the superficial lamina propria, which disrupts the epithelium (4–7) with degeneration of basal epithelial cells and the basement membrane (8). The immunological events may begin with an external or internal antigen that alters the basal epithelial cells, rendering them susceptible to a cell-mediated immune response involving activation of antigen-presenting Langerhans cells, immunocompetent keratinocytes, CD4+ T-helper–inducer cells, CD8+ T-suppressor-cytotoxic cells, and the production of cytokines, including chemokines

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(such as interleukin-2, interferon gamma and tumour necrosis factor beta) within the epithelium and within the infiltrate by activated leucocytes (2, 4, 6, 8–14).

Previous works have shown that there is altered expression of adhesion molecules in OLP (13, 15). The adhesion molecules are proteins that control the interaction between leucocytes and the endothelium, extracellular matrix or both. The integrin leucocyte function-associated antigen 1 (LFA-1) plays an important role in leucocyte adhesion to endothelium (16, 17), which expresses its ligand intercellular adhesion molecule-1 (ICAM-1) (18). ICAM-1 is a member of the immunoglobulin supergene family. Endothelial cells, monocytes and lymphocytes in normal condition weakly express ICAM-1. However cytokines may induce ICAM-1 expression in other cells which increase the interaction between cells and endothelium at inflammatory sites (16, 19). Another member of the immunoglobulin supergene family that is expressed by the endothelial cells is vascular adhesion molecule-1 (VCAM-1). VCAM-1, which helps leucocyte adhesion to wall vessels (18), is released by activated endothelial cells (20). VCAM-1 and the Selectins mediate the initial leucocyte adhesion and transmigration across the blood vessels that may be reinforced by ICAM-1 (19).

In the present study, the cellular infiltrate was characterised in lesions of patients with OLP and normal oral mucosa (NOM). Furthermore, the distribution of some adhesion molecules was identified in the same tissues in order to confirm the role of these elements in the pathogenesis of OLP.

Materials and methods

Patients

Biopsies of patients with OLP ($n = 18$) were obtained. The patients were diagnosed using established clinical, epidemiological and histopathological criteria (21). The patients were not under treatment at the time of the study. Twelve biopsies were taken from buccal mucosa, three from tongue, two from hard palate and one from gingival lesions. Biopsies of NOM from healthy individuals ($n = 10$) were obtained from preprosthetic surgery and were used as controls. All oral biopsies were embedded in OCT compound (Miles Scientific, USA), snap-frozen in liquid nitrogen and stored at -70°C prior to analysis. Cryostat numbered serial sections were cut at 5 μm and air-dried overnight before immunostaining. At least 15 sections were prepared for each sample.

Monoclonal antibodies

All monoclonal antibodies used were diluted in a modified phosphate buffered saline (PBS). For cellular characterisation, antibodies that recognised the following mononuclear cell markers were used: CD4 (MT310 at 1 : 1000) (22), CD8 (DK25 at 1 : 1000) (23), CD1a (NA1/34 at 1 : 100) (24), LFA-1 (CD11a, MHM24 at 1 : 100) (25, 26), ICAM-1 (CD54, 6.5B5 at 1 : 100) (27), and VCAM-1 (CD106, 1.4C3 at 1 : 100) (28). All the antibodies used were from DAKO.

Immunoperoxidase staining

The immunostaining procedure was carried out as previously described (29–31). Briefly, the samples were hydrated in PBS and sequentially incubated for 60 min with primary mouse monoclonal antibody, biotinylated horse antimouse IgG (Vector, USA) for 30 min, and Avidin–Biotin–Peroxidase conjugate (ABC Elite Complex, Vector) for 15 min. Three washes with PBS were performed between each incubation. The reactions were developed for 7 min with 3-amino-9-ethylcarbazole (AEC). The sections were then washed and counterstained with Mayer's haematoxylin. Negative controls included omission of the primary antibody and some were incubated with control 'irrelevant' primary antibody of identical Ig isotype.

Quantification and statistical analysis

Cells were quantified under a light microscope with a calibrated monitor to determine the number of cells/mm^2 and the information was expressed as mean ± standard error of the mean. At least 30 random microscopic fields were evaluated for each marker. Epithelial and endothelial cell staining for ICAM-1 and VCAM-1 was graded semiquantitatively using a scale previously used by Walton et al. (13), for example no staining, focal and light staining, moderate staining, intense staining. Comparison between groups was made with the non-parametric Mann–Whitney *U*-test and the parametric Student's *t*-test for unpaired samples. Any *P*-values <0.05 were considered significant.

Results

The group of OLP patients was composed of 1 male and 17 females with a mean age of 59.44 ± 11.25 years. Leucocyte cell

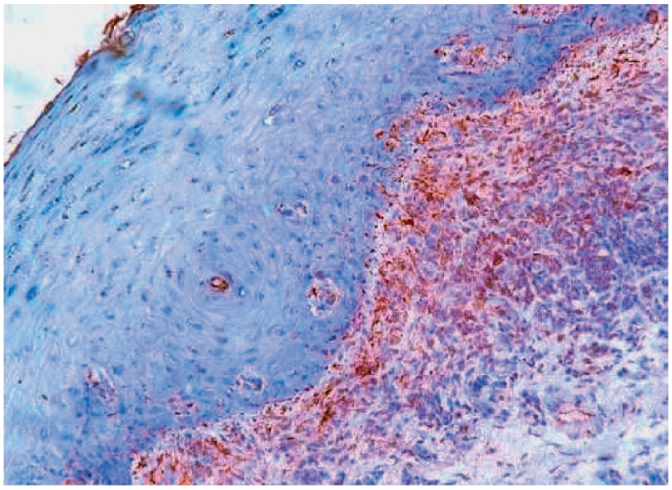

Fig. 1. Immunoreactivity for CD4+ T cells ×200.

densities were higher in OLP than in NOM. OLP patients showed a high number of CD4+ (1457 ± 195 cell/mm^2) and CD8+ T-lymphocytes (1552 ± 203 cell/mm^2) in the infiltrate towards the basal membrane (Figs. 1–3). The CD4/CD8 ratio in OLP lesions was 0.9, indicating a higher proportion of T-suppressor-cytotoxic cells. NOM showed 52 ± 9 cell/mm^2 CD4+ T cells and 75 ± 11 cell/mm^2 CD8+ T cells. The comparison of leucocyte cells between OLP and NOM was statistically significant ($P < 0.01$).

The results showed augmented numbers of epithelial dendritic CD1a+ cells (163 ± 27 cell/mm^2) in the basal keratinocyte layer in OLP. CD1a+ Langerhans cells were significantly less ($P < 0.01$) in NOM (55 ± 10 cell/mm^2).

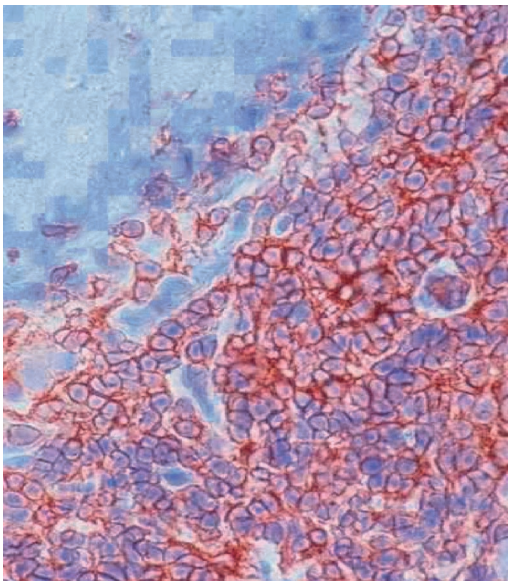

Fig. 2. Immunoreactivity for CD8+ T cells ×400.

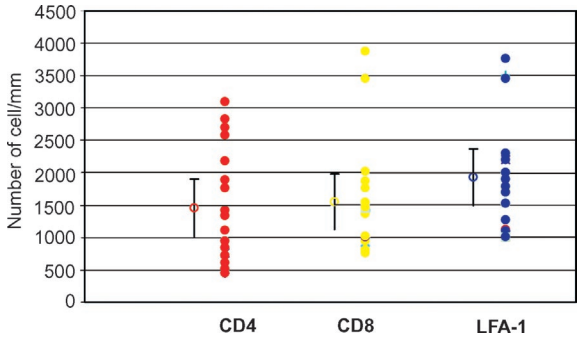

Fig. 3. Number of CD4+, CD8+ and LFA-1+ cells/mm in OLP. Solid circles represent patients. Open circeles represents means and error bars are SD.

In all OLP lesions, the T-lymphocyte infiltrate and endothelial cells expressed the adhesion molecule ICAM-1 in the lamina propria. In 10 patients, keratinocytes expressed ICAM-1, mainly in the basal layer. NOM biopsies showed only scattered ICAM-1 positive blood vessels in the lamina propria and in only 2 of 10 NOM did keratinocytes express ICAM-1 in the superficial rather than basal epithelial layers. The expression of ICAM-1 on all cell types was significantly higher in OLP lesions than that of NOM ($P < 0.05$).

Cells near the epithelium expressed the adhesion molecule LFA-1. Many LFA-1+ cells were observed near ICAM-1+ keratinocytes. The number of LFA-1+ cells was significantly higher in OLP lesions (1917 ± 231 cell/mm^2) (Fig. 3) than NOM (68 ± 18 cell/mm^2; $P < 0.01$). Serial section staining suggested LFA-1+ cells were CD4+ and CD8+ 0.

Adhesion molecule VCAM-1 was more highly expressed in OLP than in NOM ($P < 0.05$). The VCAM-1 distribution was different to ICAM-1, in OLP 9 patients' (50%) VCAM-1 was expressed by T cells in the lamina propria, and by the endothelial cells in 16 patients (88%). In 50% of NOM, the expression of VCAM-1 was restricted to a few blood vessels of the deep connective tissue.

Discussion

This study of a Venezuelan population confirms findings of previous immunopathological studies (13, 15, 32, 33). The prominent cellular infiltrate was characterised to determine the presence of several cellular immunophenotypes. OLP showed an increase in CD4+ T cells, and CD8+ T cells in accord with several other authors (4–7, 34–37). This infiltrate was mainly represented by CD8+ suppressor-cytotoxic cells. Previous

studies have showed that CD8+ T cells constitute a high proportion of the cellular infiltrate in OLP (4, 6, 7, 35). However, some studies have shown a high proportion of CD4+ T cells (36, 37). These differences may occur because of disease progression. Early lesions may show higher proportions of CD4+ cells, which promote the influx of CD8+ cells seen in older lesions.

Leucocytes were observed in NOM in proportions similar to a previous investigation (38). The CD4/CD8 ratio observed by van Loon et al. (38) indicated a high proportion of CD8+ T cells in normal conditions. These results are very similar to our observations.

Augmented numbers of Langerhans cells in OLP was observed previously (32, 34, 36, 39–41). This finding is consistent with the prevailing view that a high density of dendritic cells CD1a+ (Langerhans cells) in the epithelium of OLP has an important role in the antigen presentation and T-cell activation (42). By contrast, Sloberg et al. (39), Farthing et al. (40) and McCartan et al. (43) observed similar expression of CD1a+ in Langerhans cells in OLP and NOM. However, Class II antigen expression by Langerhans cells was found higher in OLP demonstrating an increase of Langerhans cells immunologically active (39, 40, 43, 44).

In this study, a high expression of LFA-1, VCAM-1 and ICAM-1 was observed confirming previous studies (15, 25, 34). There is a close relation between the increase of T cells and the expression of these adhesion molecules (38). Activated CD4+ and CD8+ T cells expressed LFA-1 in OLP. A clear correlation between LFA-1+ cells and keratinocytes in OLP has been shown in some studies (15, 25, 45). ICAM-1 plays an important role in T-cell activation and in the destruction of the basal keratinocyte layer in OLP lesions (46). The expression of ICAM-1 on keratinocytes could have an essential place in the antigen processing process promoting T-cell activation and keratinocytes destruction. This premise has been stated by several studies (15, 25, 43). However, Verdickt et al. (26) and Li et al. (46) suggest that ICAM-1 on keratinocytes are not required for LFA-1+ T lymphocytes and its secondary presence is due to cytokines release during the keratinocyte attack. T-cell migration toward epithelium in OLP could involve other adhesion molecules (33, 47) but it is inconclusive which are responsible for the initiation of the disease. VCAM-1 was expressed mainly on endothelial cells and seems to be indispensable in the lymphocyte–endothelium interaction. In OLP, vascular adhesion molecules are involved in the movement of T cells from blood vessels to epithelium (15, 47). VCAM-1 could be responsible for retention of cytotoxic cells near the epithelium and that typifies the disease (15).

ICAM-1 expression was weakly observed in NOM. VCAM-1 expression was only observed in a few deeper submucosal vessels contrary to Regezi et al. (15). ICAM-1 and VCAM-1 could have a role in the presence of lymphocytes in NOM. The up-regulation of ICAM-1 and VCAM-1 might be found under inflammatory conditions which augment migration of T cells.

In summary, it is suggested that in OLP there is an immune response to an exogenous or endogenous antigen found in basal keratinocytes which activates Langerhans cells. These cells present the antigen to CD4+ T lymphocytes that go to the oral mucosa lamina propria through activation of adhesion molecules. LFA-1 is expressed in T lymphocytes, ICAM-1 is expressed in endothelial cells and keratinocytes and VCAM-1 is expressed in blood vessels. Keratinocytes respond to injury by producing cytokines which promote, with CD4+ T lymphocytes and adhesion molecules, CD8+ T-lymphocyte stimulation. CD8+ cytotoxic cells are distributed near epithelium in order to destroy it.

References

1. Mollaoglu N. Oral lichen planus: a review. *Br J Oral Maxillofac Surg* 2000; **38**: 370–7.
2. Sugerman PB, Rollason PA, Savage NW, Seymour GJ. Suppresor cell function in oral lichen planus. *J Dental Res* 1992; **71**: 1916–9.
3. Femiano F, Cozzolino F, Gaeta GM, De Luca P, Perfetto B, Baroni A. Recents advances on the pathogenesis of oral lichen planus. The adhesion molecules. *Minerva Stomatol* 1999; **48**: 151–9.
4. Yamamoto T, Yoneda K, Osaki T. Cellular immunosuppression in oral lichen planus. *J Oral Pathol Med* 1990; **19**: 464–70.
5. Walsh LJ, Ishii T, Savage NW, Gemmell E, Seymour GJ. Immunohistologic analysis of epithelial cell populations in oral lichen planus. *J Oral Pathol Med* 1990; **19**: 177–81.
6. Gadenne AS, Strucke R, Dunn D, Wagner M, Bleicher P, Bigby M. T-cell lines derived from lesional skin of lichen planus patients contain a distintive population of T-cell receptor-bearing cell. *J Invest Dermatol* 1994; **103**: 347–51.
7. Becker J, Loning TH, Reichart P, Hartmann N. Oral lichen planus. characterization of immunocompetent cell with hybridoma antibodies. *J Oral Pathol* 1983; **12**: 117–23.
8. Walsh LJ, Savage NW, Ishii T, Seymour GJ. Immunopathogenesis of oral lichen planus. *J Oral Pathol Med* 1990; **19**: 389–96.
9. Daud SM, et al. Lichen planus. In: Freedberg IM, Eisen AZ, Wolff K, Austen FK, Goldsmith LA, Katz SI, Fitzpatrick TB, eds. *Dermatology in General Medicine.* USA.: Mc Graw-Hill, 1999; 561–77.
10. Yamamoto T, Nakane T, Osaki T. The mechanism of mononuclear cell infiltration in oral lichen planus: the role of cytokines released from keratinocytes. *J Clin Immunol* 2000; **20**: 294–305.
11. Yamamoto T, Osaki T, Yoneda K, Ueta E. Cytokine production by keratinocytes and mononuclear infiltrates in oral lichen planus. *J Oral Pathol Med* 1994; **23**: 309–15.
12. Becker J, Schuppan D. Altered expression of extracellular matrix proteins and integrins in oral lichen planus. *J Oral Pathol Med* 1995; **24**: 159–64.

13. Walton LJ, Thornhill MH, Farthing PM. VCAM-1 and ICAM-1 are expressed by Langerhans cells, macrophages and endothelial cells in oral lichen planus. *J Oral Pathol Med* 1994; **23**: 262–8.
14. Carrozo M, Gandolfo S, Lodi G, et al. Oral lichen planus in patients infected with hepatitis C virus: the role of autoinmunity. *J Oral Pathol Med* 1999; **28**: 16–9.
15. Regezi JA, Dekker NP, Macphail LA, Lozada-Nur F, Mccalmont TH. Vascular adhesion molecules in oral lichen planus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1996; **81**: 682–90.
16. Springer TA. Adhesion receptors of the immune system. *Nature* 1990; **346**: 425–34.
17. Clark E, Brugget J. Integrins and Signal transduction Pathways. *The Road Taken Sci* 1995; **268**: 233–8.
18. Marlin SD, Springer TA. Purified Intercellular Adhesion Molecule-1 (ICAM-1) is a Ligand for Lymphocyte Function-Associatied Antigen 1 (LFA-1). *Cell* 1987; **51**: 813–9.
19. Springer TA. Traffic signal for lymphocyte recirculation and leukocyte emigration. *The Multistep Paradigm Cell* 1994; **76**: 301–14.
20. Gearing AJH, Newman W. Circulating adhesion molecules in disease. *Immunol Today* 1993; **14**: 506–12.
21. Regezi JA, Sciubba JJ. *Oral Pathology: Clinical Pathologic Correlation*. Philadelphia: W.B. Saunders Company, 1999; 101–6.
22. Stein H, Lennert K, Feller AC, Mason DY. Immunohistological analysis of human lymphoma: correlation of histological and immunological categories. *Adv Cancer Res* 1984; **42**: 67–147.
23. Ledbeher JA, Evans RI, Lipinski M, Cunningham-Rudles C, Good RA, Herzenberg LA. Evolutionary conservation of surface molecules that distinguish T-lymphocyte helper/inducer and cytotoxic/suppressor subpopulation in mouse and man. *J Exp Med* 1981; **153**: 310–23.
24. Mcmichael AJ, Pilch JR, Galfré G, Mason DY, Fabre JW, Milstein C. A human thymocyte antigen defined by a hybrid meyloma monoclonal antibody. *Eur J Immunol* 1979; **9**: 205–10.
25. Eversole LR, Dam J, Ficarra G, Hwang CY. Leukocyte adhesion molecules in oral lichen planus: a T cell-mediated immunopathologic process. *Oral Microbiol Immunol* 1994; **9**: 376–83.
26. Verdickt GM, Savage NW, Dodd NM, Walsh LJ. Expression of the CD54 (ICAM-1) and CD11a (LFA-1) adhesion molecules in oral mucosal inflammation. *J Oral Pathol Med* 1992; **21**: 65–9.
27. Garioch JJ, MacKie RM, Campbell I, Forsyth A. Keratinocyte expression of intercellular adhesion molecule-1 (ICAM-1) correlated with infiltration of lymphocyte function associated antigen 1 (LFA-1) positive cells in evolving allergic contact dermatitis reactions. *Histopathology* 1991; **19**: 351–4.
28. Wilkinson LS, Edwards JCW, Poston RN, Haskard DO. Expression of vascular cell adhesion molecule-1 in normal and inflamed synovium. *Lab Invest* 1993; **68**: 82–8.
29. Hsu SM, Raine L, Fanger H. Use of Avidin–Biotin–Peroxidase complex (ABC) in Immunoperoxidase techniques. *J Histochem Cytochem* 1981; **29**: 577–80.
30. Tapia FJ, Caceres-Dittmar G, Sanchez MA, Fernandez CT, Rondon AJ, Convit J. Adhesion molecules in lesions of American cutaneous leishmaniasis. *Exp Dermatol* 1994; **3**: 17–22.
31. Martinez-Arends A, Tapia FJ, Caceres-Dittmar G, Mosca W, Valecillos L, Convit J. Immunocytochemical characterization of immune cells in lesion of American cutaneus leishmaniasis using novel T-cell markers. *Acta Tropica* 1991; **49**: 271–80.
32. Regezi JA, Stewart JC, Lloyd RV, Headington JT. Immunohistochemical staining of Langerhans cells and macrophages in oral lichen planus. *Oral Surg Oral Med Oral Pathol* 1985; **60**: 396–402.
33. Walton LJ, Thornhill MH, MacEy MG, Farthing PM. Cutaneous lymphocyte associated antigen (CLA) and αeβ7 integrins are expressed by mononuclear cells in skin and oral lichen planus. *J Oral Pathol Med* 1997; **26**: 402–7.
34. Walton LJ, MacEy MG, Thornhill MH, Farthing PM. Intra-epithelial subpopulations of T lymphocytes and Langerhans cells in oral lichen planus. *J Oral Pathol Med* 1998; **27**: 116–23.
35. Sugerman PB, Voltz MJ, Savage NW, Basford KE, Seymour GJ. Phenotypic and functional analysis of peripheral blood lymphocytes in oral lichen planus. *J Oral Pathol Med* 1993; **21**: 445–50.
36. Boisnic S, Frances C, Branchet MC, Szpirglas H, Charpentier YL. Immunohistochemical study of oral lesions of lichen planus. *Oral Surg Oral Med Oral Pathol* 1990; **70**: 462–5.
37. Bhan AK, Harrist TJ, Murphy GF, Mihn MC Jr. T-cell subsets and Langerhans cells in lichen planus; *in situ* characterization using monoclonal antibodies. *Br J Dermatol* 1981; **105**: 617–21.
38. van Loon LAJ, Krieg SR, Davidson CL, Bos JD. Quantification and distribution of lymphocyte subsets and Langerhans cells in normal human oral mucosa and skin. *J Oral Pathol Med* 1989; **18**: 197–201.
39. Sloberg K, Jonsson R, Jontell M. Assessment of Langerhans' cells in oral lichen planus using monoclonal antibodies. *J Oral Pathol* 1984; **13**: 516–24.
40. Farthing PM, Matear P, Cruchley AT. The activation of Langerhans cells in oral lichen planus. *J Oral Pathol Med* 1990; **19**: 81–5.
41. Farthing PM, Matear P, Cruchley AT. Langerhans cell distribution and keratinocyte expression of HLADR in oral lichen planus. *J Oral Pathol Med* 1992; **21**: 451–5.
42. Pitagala-Arachi A, Crane IJ, Scully C, Prime SS. Epithelial dendritic cells in pathological human tissues. *J Oral Pathol Med* 1989; **18**: 11–6.
43. McCartan BE, Lamey PJ. Expression of CD1 and HLA-DR by Langerhans Cells in oral lichenoid drug eruptions and idiopathic oral lichen planus. *J Oral Pathol Med* 1997; **26**: 176–80.
44. Ishii T. Immunohistochemical demonstration of T-cell subsets and accessory cells in oral lichen planus. *J Oral Pathol Med* 1987; **16**: 356–61.
45. Konter U, Kellner I, Hoffmeister B, Sterry W. Induction and upregulation of adhesion receptors in oral and dermal lichen planus. *J Oral Pathol Med* 1990; **19**: 459–63.
46. Li J, Mahiouz DL, Farthing PM, Haskard DO, Thornhill MH. Heterogeneity of ICAM-1 expression, and cytokine regulation of ICAM-1 expression, in skin and oral keratinocytes. *J Oral Pathol Med* 1996; **25**: 112–8.
47. Kirby AC, Olsen I, Farthig PM, Porter SR. Expression of lymphocyte function-associated antigen 3 in oral lichen planus. *Oral Dis* 1995; **1**: 193–7.

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