Significantly higher frequencies of presence of serum autoantibodies in Chinese patients with oral lichen planus

Julia Yu-Fong Chang^{1,2}, Chun-Pin Chiang^{1,2}, Chuhsing Kate Hsiao³, Andy Sun^{1,2}

¹School of Dentistry, College of Medicine, National Taiwan University, Taipei, Taiwan; ²Department of Dentistry, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan; ³Department of Public Health, College of Public Health, National Taiwan University, Taipei, Taiwan

BACKGROUND: Previous studies have shown the presence of serum anti-nuclear (ANA), anti-smooth muscle (SMA), anti-mitochondrial (AMA), anti-gastric parietal cell (GPCA), anti-thyroglobulin (TGA) and anti-thyroid microsomal autoantibodies (TMA) in small groups of patients with oral lichen planus (OLP).

METHODS: In this study, the serum levels of ANA, SMA, AMA, GPCA, TGA and TMA were measured in a group of 320 Chinese OLP patients and 53 healthy control subjects to assess whether Chinese OLP patients had significantly higher frequencies of serum autoantibodies than healthy control subjects and to assess which risk factors had a significant influence on the possession of a specific serum autoantibody in OLP patients.

RESULTS: We found that autoantibodies were present in 195 (60.9%) of the 320 OLP patients. The frequencies of presence of serum ANA (28.1%), GPCA (26.3%), TGA (21.3%) and TMA (24.4%) in OLP patients were significantly higher than those (5.7%, 1.9%, 1.9% and 1.9%, respectively) in healthy control subjects (all *P*-values were < 0.005). Forty-one (12.8%) OLP patients also had anti-hepatitis C virus antibody (HCVA) in their sera. The multivariate logistic regression found that major erosive OLP (EOLP) [odds ratio (OR) = 1.786], TGA/TMA-positivity (OR = 2.517), and HCVA-positivity (OR = 2.214) were significant risk factors to influence ANA-positivity in OLP patients. Moreover, only major EOLP (OR = 1.879) and ANA-positivity (OR = 2.581) were significant risk factors to influence TGA/TMA-positivity in OLP patients.

CONCLUSIONS: There are significantly higher frequencies of presence of ANA, GPCA, TGA and TMA in Chinese OLP patients than in healthy control subjects.

J Oral Pathol Med (2009) 38: 48–54

Keywords: anti-hepatitis C virus antibody; autoantibodies; oral lichen planus

Introduction

Oral lichen planus (OLP) is a chronic inflammatory oral mucosal disease. Previous study showed involvement of both antigen-specific and non-specific mechanisms in OLP. Antigen-specific mechanisms include antigen presentation by basal keratinocytes and antigen-specific keratinocyte killing by CD8⁺ cytotoxic T lymphocytes. Non-specific mechanisms include mast cell degranulation and matrix metalloproteinase activation in OLP lesions (1). Through mast cell/T-cell interactions in OLP lesions, mast cell-released cytokines, chemokines and matrix metalloproteinases can promote T-cell activation, migration, proliferation and differentiation (2). OLP is histologically characterized by liquefaction degeneration of basal epithelial cells and an intraepithelial and subepithelial infiltrate of mononuclear cells which are predominantly CD8⁺. CD4⁺ cells are observed mainly in the deep lamina propria (3). An increase in histocompatibility leukocyte antigen (HLA)-D-related (DR)-positive CD3⁺ cells in both the local lesional tissues and peripheral lymphocytes also indicates T-cell activation in OLP (4, 5). The above findings suggest that OLP is a T-cell-mediated inflammatory disease.

Previous studies have shown the presence of serum autoantibodies including anti-epithelial cell, anti-nuclear (ANA), anti-smooth muscle (SMA), anti-mitochondrial (AMA), anti-gastric parietal cell (GPCA), anti-thyroglobulin (TGA), anti-thyroid microsomal (TMA) and anti-desmogleins 1 and 3 antibodies in small groups of OLP patients (all studies *n* < 33 except ours *n* = 63) (6–11). In this study, we examined the frequencies of presence of serum autoantibodies including ANA, SMA, AMA, GPCA, TGA and TMA in a large group of 320 Chinese OLP patients and in 53 healthy control subjects. We tried to find whether there were significantly higher frequencies of presence of serum autoantibodies in Chinese OLP patients than in healthy control subjects. In addition, we also used univariate and multivariate analyses to assess which risk factors had a significant influence on the possession of a specific serum autoantibody in OLP patients.

Correspondence: Dr Andy Sun, Department of Dentistry, National Taiwan University Hospital, 1 Chang-Te Street, Taipei 10048, Taiwan. Tel: +886 2 2312 3456 (ext. 7702), Fax: +886 2 2389 3853, E-mail: andysun7702@yahoo.com.tw
Accepted for publication April 22, 2008

Materials and methods

Subjects

The study group consisted of 320 OLP patients (53 men and 267 women, age range 21–83 years, mean 55.3 years) without LP of other mucosal or skin surfaces. The normal control group consisted of 53 healthy subjects (eight men and 45 women, age range 21–83 years, mean 54.7 years) without any oral mucosal or systemic diseases. All the patients and control subjects were seen consecutively, diagnosed, and treated in the Department of Oral Diagnosis of National Taiwan University Hospital from July 2004 to June 2007. OLP patients with areca quid chewing habit, hypertension and autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, pemphigus vulgaris and cicatricial pemphigoid were excluded. In addition, none of them had taken any prescription medication at least 3 months before entering the study. The 320 OLP patients included 292 (50 men and 242 women, age range 21–83 years, mean 55.5 years) with erosive OLP (EOLP) and 28 (three men and 25 women, age range 24–83 years, mean 52.8 years) with non-erosive OLP (NEOLP). They were selected according to the following criteria: (i) a typical clinical presentation of radiating grayish-white Wickham striae, papules and plaques, separately or in combination (NEOLP), and erosion or ulceration on the oral mucosa (EOLP) and (ii) biopsy specimens characteristic of OLP, that is, hyperkeratosis or parakeratosis, a slightly acanthotic epithelium with liquefaction degeneration of the basal epithelial cells, a pronounced band-like lymphocytic infiltrate in the lamina propria, and the absence of epithelial dysplasia. The EOLP was further divided into the major and minor types according to criteria described previously (12). The blood samples were drawn from OLP patients and healthy control subjects. The serum autoantibody, anti-hepatitis C virus antibody (HCVA) and blood glucose levels were determined. This study was reviewed and approved by the Human Investigation Review Committee at the National Taiwan University Hospital.

Determination of serum autoantibody levels

The circulating ANA, SMA, AMA and GPCA were detected by the indirect immunofluorescence technique with HEp-2 cells (Medical & Biological Laboratories Co., Nagaya, Japan; for ANA), rat kidney (for SMA and AMA) and rat stomach (for GPCA) as substrates as described previously (10, 13). In brief, HEp-2 cells or 5 µm-thick cryostat sections of substrate tissues on slides were reacted with serially diluted OLP patients' and control subjects' sera in a moist chamber at room temperature for 30 min. The initial dilution of the patients' and control subjects' sera was 1:20 with PBS. After washing, the sections were incubated with fluorescein isothiocyanate (FITC)-labeled goat anti-human IgG antiserum (Boehringer Mannheim Biochemicals, Indianapolis, IN, USA) which had been pre-diluted and kept in dropper vial by the manufacturer and was ready-to-use for another 30 min. The sections were washed

again, mounted with buffered glycerine, and examined with an Olympus fluorescence microscope (Olympus, Tokyo, Japan). Sera were scored as positive when they produced fluorescence at a dilution of 160-fold or more for ANA, and of 20-fold or more for SMA, AMA and GPCA.

TGA and TMA titers were measured by a semi-quantitative microtiter particle agglutination test using Serodia-AMC kits (Fujirebio Inc., Tokyo, Japan) as described previously (10, 13). In brief, serial 2-fold dilutions of initial 1:20 dilutions of OLP patients' and control subjects' sera in the wells of a microtitration tray were reacted with gelatin particles sensitized with thyroglobulin or thyroid microsomal antigen at room temperature for 3 hr. The results were obtained by reading the settling patterns of the colored gelatin particles using a tray viewer. Serum containing TGA or TMA reacted with the thyroglobulin or thyroid microsomal antigen-sensitized colored gelatin particles to form a smooth mat of agglutinated particles in the microtitration tray. Negative reactions were characterized by a compact button formed by the settling of the non-agglutinated particles. TGA or TMA titers equal to or greater than 1:40 were considered positive.

Determination of HCVA level

The serum HCVA was detected by an ARCHITECT anti-HCV assay as described previously (14, 15). This assay was an automated chemiluminescent microparticle immunoassay (CMIA) based on the enzyme immunoassay (EIA) principle. In contrast to the EIA, where the antigen-antibody complexes were detected by enzyme-labeled conjugate, the CMIA used acridinium labeled conjugate as detection system. The resulting chemiluminescent reaction was measured as relative light units (RLUs). ARCHITECT Anti-HCV was designed to detect antibodies to structural and non-structural proteins of the HCV genome (NS3, NS4, core). The ARCHITECT Anti-HCV assay cutoff value was calculated by the following formula: calibrator 1 mean RLU value $\times$ 0.074 = cutoff RLU. ARCHITECT delivered specimen results as a ratio of the specimen signal (in RLU) to the cutoff value (S/CO). S/CO ratios of ≥ 1.00 were considered reactive for HCVA; ratios less than 1.00 were non-reactive for HCVA.

Diagnosis of diabetes mellitus

Patients with fasting plasma glucose levels more than 126 mg/ml on two or more tests on different days or a random blood glucose level of 200 mg/ml or higher were diagnosed as having diabetes mellitus (DM).

Statistical analysis

The difference in frequency of presence of each serum autoantibody was compared between any two groups by chi-square or Fisher's exact test, where appropriate. The multivariate logistic regression was used to assess which risk factors including the age, gender, OLP type (EOLP or NEOLP), EOLP type (major or minor), and the presence of other autoantibodies, HCVA or DM had a

significant influence on the possession of a specific serum autoantibody in OLP patients. The result was considered to be significant if the P -value was less than 0.05.

Results

Autoantibodies were present in 195 (60.9%) of the 320 OLP patients: 104 (32.5%) had only one autoantibody, 44 (13.7%) two autoantibodies, 30 (9.4%) three autoantibodies, 16 (5.0%) four autoantibodies and 1 (0.3%) five autoantibodies. The frequencies of presence of ANA, SMA, AMA, GPCA, TGA and TMA in different groups of OLP patients and 53 healthy control subjects are shown in Table 1. We found that the frequencies of presence of serum ANA (28.1%, $P = 0.000$), GPCA (26.3%, $P = 0.000$), TGA (21.3%, $P = 0.002$) and TMA (24.4%, $P = 0.000$) in OLP patients were significantly higher than the corresponding autoantibody frequencies (5.7%, 1.9%, 1.9% and 1.9%, respectively) in healthy control subjects. If the OLP patients were further divided into two subgroups according to the age, gender, and the presence of erosive lesion (EOLP or NEOLP), HCVA or DM, all subgroups of OLP patients also had significantly higher frequencies of presence of serum ANA, GPCA, TGA and TMA than healthy control subjects except the

frequency of serum ANA in OLP patients ≤ 50 years of age ($P = 0.059$, marginal significance) and the frequency of serum TGA in male OLP patients ($P = 0.118$, Table 1).

Of 320 OLP patients, 41 (12.8%) were also HCVA-positive and 47 (14.7%) also had DM (Table 1). Univariate analysis by chi-squared test showed a significant correlation between ANA-positivity and OLP patients with the age > 50 years ($P = 0.008$), major EOLP ($P = 0.000$), or the presence of GPCA ($P = 0.026$), TGA/TMA ($P = 0.000$), or HCVA ($P = 0.000$) in their sera (Table 2). The multivariate logistic regression found that major EOLP [odds ratio (OR) = 1.786, $P = 0.033$], TGA/TMA-positivity (OR = 2.517, $P = 0.001$) and HCVA-positivity (OR = 2.214, $P = 0.030$) were significant risk factors to influence ANA-positivity in OLP patients.

The GPCA-positivity in OLP patients was significantly associated with the presence of serum ANA ($P = 0.026$) or TGA/TMA ($P = 0.026$, Table 3), whereas neither of these two risk factors reached the significant level (both P -values = 0.070) to influence the GPCA-positivity in OLP patients by multivariate logistic regression. Furthermore, there was a significant correlation between the TGA/TMA-positivity and major EOLP ($P = 0.000$) or the presence of ANA

Table 1 Frequencies of presence of serum ANA, SMA, AMA, GPCA, TGA and TMA antibodies in different groups of OLP patients and 53 healthy control subjects

	Autoantibodies (positive patient number, %)					
	ANA	SMA	AMA	GPCA	TGA	TMA
OLP ($n = 320$)	90 (28.1)	27 (8.4)	5 (1.6)	84 (26.3)	68 (21.3)	78 (24.4)
P -value ^a	0.000	0.056	0.786	0.000	0.002	0.000
≤ 50 years ($n = 105$)	19 (18.1)	10 (9.5)	2 (1.9)	24 (22.9)	20 (19.0)	17 (16.2)
P -value ^a	0.059	0.048	0.797	0.001	0.006	0.016
> 50 years ($n = 215$)	71 (33.0)	17 (7.9)	3 (1.4)	60 (27.9)	48 (22.3)	61 (28.4)
P -value ^a	0.000	0.072	0.892	0.000	0.001	0.000
Male ($n = 53$)	13 (24.5)	4 (7.5)	0 (0)	14 (26.4)	6 (11.3)	8 (15.1)
P -value ^a	0.015	0.126	–	0.000	0.118	0.037
Female ($n = 267$)	77 (28.8)	23 (8.6)	5 (1.9)	70 (26.2)	62 (23.2)	70 (26.2)
P -value ^a	0.000	0.054	0.691	0.000	0.000	0.000
EOLP ($n = 292$)	83 (28.4)	24 (8.2)	5 (1.7)	78 (26.7)	62 (21.2)	72 (24.7)
P -value ^a	0.009	0.061	0.738	0.000	0.002	0.000
NEOLP ($n = 28$)	7 (25.0)	3 (10.7)	0 (0)	6 (21.4)	6 (21.4)	6 (21.4)
P -value ^a	0.031	0.070	–	0.006	0.006	0.006
HCVA (+) ($n = 41$)	21 (51.2)	7 (17.1)	1 (2.4)	14 (34.1)	12 (29.3)	16 (39.0)
P -value ^a	0.000	0.006	0.897	0.000	0.000	0.000
HCVA (–) ($n = 279$)	69 (24.7)	20 (7.2)	4 (1.4)	70 (25.1)	56 (20.1)	62 (22.2)
P -value ^a	0.004	0.090	0.849	0.000	0.003	0.001
DM (+) ($n = 47$)	15 (31.9)	2 (4.3)	1 (2.1)	16 (34.0)	7 (14.9)	12 (25.5)
P -value ^a	0.002	0.423	0.952	0.000	0.043	0.001
DM (–) ($n = 273$)	75 (27.5)	25 (9.2)	4 (1.5)	68 (24.9)	61 (22.3)	66 (24.2)
P -value ^a	0.001	0.044	0.838	0.000	0.001	0.000
EOLP						
Major ($n = 128$)	50 (39.1)	9 (7.0)	3 (2.3)	41 (32.0)	40 (31.3)	43 (33.6)
P -value ^a	0.000	0.109	0.628	0.000	0.000	0.000
Minor ($n = 164$)	33 (20.1)	15 (9.1)	2 (1.2)	37 (22.6)	22 (13.4)	29 (17.7)
P -value ^a	0.025	0.049	0.985	0.001	0.035	0.008
Healthy controls ($n = 53$)	3 (5.7)	0 (0)	0 (0)	1 (1.9)	1 (1.9)	1 (1.9)

HCVA, anti-hepatitis C virus antibody; DM, diabetes mellitus; OLP, oral lichen planus; EOLP, erosive OLP; NEOLP, non-erosive OLP; ANA, anti-nuclear antibody; SMA, anti-smooth muscle antibody; AMA, anti-mitochondrial antibody; GPCA, anti-gastric parietal cell antibody; TMA; anti-thyroid microsomal antibodies; TGA, anti-thyroglobulin antibody.

^aComparison between healthy control and any other groups by chi-squared or Fisher's exact test, where appropriate.

Table 2 Univariate analysis of correlation between the presence of ANA and other affecting factors in OLP patients

	ANA (+) (n = 90)	ANA (-) (n = 230)	P-value (chi-squared test)
Age			0.008
≤ 50	19	86	
> 50	71	144	
Gender			0.638
Male	13	40	
Female	77	190	
OLP type			0.869
EOLP	83	209	
NEOLP	7	21	
EOLP type			0.000
Major	50	78	
Minor	33	131	
GPCA			0.026
Positive	32	52	
Negative	58	178	
TGA or TMA			0.000
Positive	44	53	
Negative	46	177	
SMA			0.394
Positive	10	17	
Negative	80	213	
AMA			0.925
Positive	2	3	
Negative	88	227	
HCVA			0.000
Positive	21	20	
Negative	69	210	
DM			0.653
Positive	15	32	
Negative	75	198	

HCVA, anti-hepatitis C virus antibody; DM, diabetes mellitus; OLP, oral lichen planus; EOLP, erosive OLP; NEOLP, non-erosive OLP; ANA, anti-nuclear antibody; SMA, anti-smooth muscle antibody; AMA, anti-mitochondrial antibody; GPCA; anti-gastric parietal cell antibody; TMA; anti-thyroid microsomal antibodies; TGA, anti-thyroglobulin antibody.

($P = 0.000$), GPCA ($P = 0.026$) or HCVA ($P = 0.027$) in OLP patients (Table 4). The multivariate logistic regression found that only major EOLP (OR = 1.879, $P = 0.016$) and ANA-positivity (OR = 2.581, $P = 0.001$) were significant risk factors to influence TGA/TMA-positivity in OLP patients. However, the presence of SMA or AMA in OLP patients was not related to the affecting factors such as the age, gender, OLP type, EOLP type, and the presence of other autoantibodies, HCVA or DM in OLP patients (Tables 5 and 6).

We further calculated the overlap of serum autoantibodies in 320 OLP patients. If 320 OLP patients were divided into three subgroups according to the possession of organ-specific (GPCA or TGA/TMA) or non-organ-specific (ANA/SMA/AMA) serum autoantibodies: 84 (26.3%) had GPCA, 97 (30.3%) had TGA/TMA and 110 (34.4%) had ANA/SMA/AMA in their sera. Of 84 GPCA-positive OLP patients, 34 (40.5%) were also TGA/TMA-positive and 36 (42.9%) ANA/SMA/AMA-positive. Of 97 TGA/TMA-positive OLP patients, 34 (35.1%) were also GPCA-positive and 49 (50.5%) ANA/SMA/AMA-positive. Furthermore, of 110 ANA/SMA/AMA-positive OLP patients, 36 (32.7%) also had

Table 3 Univariate analysis of correlation between the presence of GPCA and other affecting factors in OLP patients

	GPCA (+) (n = 84)	GPCA (-) (n = 236)	P-value (chi-squared test)
Age			0.407
≤ 50	24	81	
> 50	60	155	
Gender			0.888
Male	14	39	
Female	70	197	
OLP type			0.702
EOLP	78	214	
NEOLP	6	22	
EOLP type			0.093
Major	41	87	
Minor	37	127	
ANA			0.026
Positive	32	58	
Negative	52	178	
TGA or TMA			0.026
Positive	34	63	
Negative	50	173	
SMA			0.788
Positive	6	21	
Negative	78	215	
AMA			0.848
Positive	1	4	
Negative	83	232	
HCVA			0.298
Positive	14	27	
Negative	70	209	
DM			0.256
Positive	16	31	
Negative	68	205	

HCVA, anti-hepatitis C virus antibody; DM, diabetes mellitus; OLP, oral lichen planus; EOLP, erosive OLP; NEOLP, non-erosive OLP; ANA, anti-nuclear antibody; SMA, anti-smooth muscle antibody; AMA, anti-mitochondrial antibody; GPCA; anti-gastric parietal cell antibody; TMA; anti-thyroid microsomal antibodies; TGA, anti-thyroglobulin antibody.

GPCA and 49 (44.5%) also had TGA/TMA in their sera.

Discussion

Previous studies demonstrated an overall incidence of 27–82% for the presence of serum autoantibodies in small groups of OLP or EOLP patients (6–11). Lin et al. (6) showed the presence of serum anti-basal cell antibodies (anti-BCA) in 54% (34/63) OLP patients. There is a decrease in the serum anti-BCA titers in six of eight anti-BCA-positive OLP patients after topical application of triamcinolone. Sun et al. (7) observed the disappearance of serum anti-BCA in three of six anti-BCA-positive EOLP patients and the disappearance of serum ANA in three EOLP patients after levamisole treatment. Lundstrom (8) demonstrated the presence of serum autoantibodies (rheumatoid factor, ANA and SMA) in 27% of the 30 OLP patients and in 9% of the 23 control subjects. Lodi et al. (9) observed the presence of circulating antibodies to epithelial antigen in 57% of 14 HCVA-positive OLP patients but in none of 14 HCVA-negative OLP patients. Carrozzo et al. (10) discovered the presence of serum

Table 4 Univariate analysis of correlation between the presence of TGA or TMA and other affecting factors in OLP patients

	<i>TGA or TMA (+) (n = 97)</i>	<i>TGA or TMA (-) (n = 223)</i>	<i>P-value (chi-squared test)</i>
Age			0.168
≤ 50	26	79	
> 50	71	144	
Gender			0.069
Male	10	43	
Female	87	180	
OLP type			0.671
EOLP	90	202	
NEOLP	7	21	
EOLP type			0.000
Major	53	75	
Minor	37	127	
ANA			0.000
Positive	44	46	
Negative	53	177	
GPCA			0.026
Positive	34	50	
Negative	63	173	
SMA			0.565
Positive	10	17	
Negative	87	206	
AMA			0.988
Positive	2	3	
Negative	95	220	
HCVA			0.027
Positive	19	22	
Negative	78	201	
DM			0.797
Positive	13	34	
Negative	84	189	

HCVA, anti-hepatitis C virus antibody; DM, diabetes mellitus; OLP, oral lichen planus; EOLP, erosive OLP; NEOLP, non-erosive OLP; ANA, anti-nuclear antibody; SMA, anti-smooth muscle antibody; AMA, anti-mitochondrial antibody; GPCA, anti-gastric parietal cell antibody; TMA; anti-thyroid microsomal antibodies; TGA, anti-thyroglobulin antibody.

autoantibodies including ANA, SMA, AMA, GPCA, anti-thyroid antibody in 41% of the 27 HCVA-positive OLP patients and in 52% of 23 HCVA-negative OLP patients. Recently, Lukac et al. (11) demonstrated significantly higher concentrations of circulating autoantibodies to both desmogleins 1 and 3 in 32 EOLP patients than in 50 healthy controls or in 25 patients with reticular OLP by enzyme-linked immunosorbent assay. Indirect immunofluorescence also revealed significantly higher frequencies of presence of both desmoglein autoantibodies in EOLP patients (82%, 18/22) than in healthy controls (5%, 1/20) or in patients with reticular OLP (20%, 3/15). This study showed an overall incidence of 60.9% for the presence of serum autoantibodies in a large group of 320 OLP patients, significantly higher frequencies of presence of serum ANA, GPCA, TGA and TMA in OLP patients than in healthy control subjects, and incidences of approximately 33–51% overlap of organ-specific and non-organ-specific serum autoantibodies in Chinese OLP patients with one or more organ-specific or non-organ-specific serum autoantibodies. The above findings suggest that there are high frequencies of presence of serum

Table 5 Univariate analysis of correlation between the presence of SMA and other affecting factors in OLP patients

	<i>SMA (+) (n = 27)</i>	<i>SMA (-) (n = 293)</i>	<i>P-value (chi-squared test)</i>
Age			0.784
≤ 50	10	95	
> 50	17	198	
Gender			0.988
Male	4	49	
Female	23	244	
OLP type			0.922
EOLP	24	268	
NEOLP	3	25	
EOLP type			0.661
Major	9	119	
Minor	15	149	
ANA			0.394
Positive	10	80	
Negative	17	213	
GPCA			0.788
Positive	6	78	
Negative	21	215	
TGA or TMA			0.565
Positive	10	87	
Negative	17	206	
AMA			0.899
Positive	0	5	
Negative	27	288	
HCVA			0.067
Positive	7	34	
Negative	20	259	
DM			0.405
Positive	2	45	
Negative	25	248	

HCVA, anti-hepatitis C virus antibody; DM, diabetes mellitus; OLP, oral lichen planus; EOLP, erosive OLP; NEOLP, non-erosive OLP; ANA, anti-nuclear antibody; SMA, anti-smooth muscle antibody; AMA, anti-mitochondrial antibody; GPCA; anti-gastric parietal cell antibody; TMA; anti-thyroid microsomal antibodies; TGA, anti-thyroglobulin antibody.

autoantibodies in OLP patients and these autoantibodies may disappear after corticosteroid or immunomodulator treatment.

The mechanisms for the induction and production of various autoantibodies in the sera of OLP patients are still not clear. The histology of OLP is characterized by liquefaction degeneration of basal epithelial cells and a subepithelial band-like infiltrate of majorly T lymphocytes and minorly B lymphocytes and macrophages (16). T-cell activation in OLP has been indirectly proven by the increase in HLA-DR-positive CD3⁺ cells in both the local lesional tissues (4) and peripheral lymphocytes (5). Moreover, a significant increase of Langerhans cells is found in the epithelium and in the lamina propria of OLP lesion compared with normal oral mucosa (17). The nuclear antigens released from the damaged basal epithelial cells might be modified by some unknown agents from the environment and phagocytosed by the Langerhans cells, macrophages and B cells. The altered autoantigens were further processed by the antigen-presenting cells and presented to the helper/inducer T cells in OLP patients' oral mucosa, regional lymph nodes and blood circulation. By the help of T cells, the antigen-specific activated B cells thus produced high

Table 6 Univariate analysis of correlation between the presence of AMA and other affecting factors in OLP patients

	AMA (+) (n = 5)	AMA (-) (n = 315)	P-value (chi-squared test)
Age			0.893
≤ 50	2	103	
> 50	3	212	
Gender			0.691
Male	0	53	
Female	5	262	
OLP type			0.621
EOLP	5	287	
NEOLP	0	28	
EOLP type			0.779
Major	3	125	
Minor	2	162	
ANA			0.925
Positive	2	88	
Negative	3	227	
GPCA			0.848
Positive	1	83	
Negative	4	232	
TGA or TMA			0.988
Positive	2	95	
Negative	3	220	
SMA			0.899
Positive	0	27	
Negative	5	288	
HCVA			0.850
Positive	1	40	
Negative	4	275	
DM			0.765
Positive	1	46	
Negative	4	269	

HCVA, anti-hepatitis C virus antibody; DM, diabetes mellitus; OLP, oral lichen planus; EOLP, erosive OLP; NEOLP, non-erosive OLP; ANA, anti-nuclear antibody; SMA, anti-smooth muscle antibody; AMA, anti-mitochondrial antibody; GPCA, anti-gastric parietal cell antibody; TMA, anti-thyroid microsomal antibodies; TGA, anti-thyroglobulin antibody.

levels of ANA in the local tissues and blood circulation of OLP patients. The locally produced autoantibodies in the interstitial fluid might diffuse into blood capillaries or be drained into the lymphatic vessels, which finally reached the blood circulation.

This study showed that major EOLP, HCVA-positivity and TGA/TMA-positivity were the three major risk factors contributing to the possession of serum ANA in OLP patients. Major EOLP lesions have greater destruction of lesional oral mucosa and thus may release more nuclear antigens into the local tissues and blood circulation than minor EOLP or NEOLP lesions. In addition, HCVA-positive and TGA/TMA-positive OLP patients may have underlying chronic liver and autoimmune thyroid diseases, respectively. Therefore, they may have more nuclear antigens released in the local liver and thyroid tissues and blood circulation. By the similar mechanisms, these nuclear antigens may stimulate the antigen-specific activated B cells to produce more ANA. The locally secreted ANA together with the ANA produced in the blood circulation finally gave rise to significantly higher levels of serum ANA in OLP patients than in healthy control subjects. The

disappearance of the anti-BCA antibodies and ANA after treatment-induced healing of the OLP lesions (6, 7) also suggests that the principal autoantigens come from the local OLP lesions.

Our previous study showed significantly higher frequencies of presence of HLA-DRw9 and HLA-Te22 antigens in Chinese OLP patients (18) and a significant association of HLA-Te22 with ANA in Chinese EOLP patients (19). HLA-DRw9 has been found to have intimate relationship with autoimmune diseases such as Graves' disease, Hashimoto's thyroiditis, myasthenia gravis and insulin-dependent DM in Chinese (20–23). HLA-DRw9 in Chinese is even considered to take the place of HLA-DR3 in Caucasians in carrying the gene which predispose to the development of autoimmune diseases (18, 21). Concomitant presence of OLP and underlying autoimmune thyroid disease trait in HLA-DRw9-positive Chinese patients may contribute to the possession of serum TGA or TMA in some of our OLP patients. In addition, an overlap of serum autoantibodies in the same individual is a common feature in patients with autoimmune disease. This study also showed an overlap of organ-specific and non-organ-specific serum autoantibodies in approximately 33–51% of OLP patients. These may partially explain why our OLP patients also had serum GPCA and why approximately 28% of our OLP patients have two or more kinds of autoantibody in their sera.

In conclusion, there are significantly higher frequencies of presence of ANA, GPCA, TGA and TMA in Chinese OLP patients. Major EOLP, TGA/TMA-positivity, and HCVA-positivity are significant risk factors to influence ANA-positivity in OLP patients. In addition, major EOLP and ANA-positivity had a significant influence on the possession of serum TGA/TMA in OLP patients.

References

1. Sugerman PB, Savage NW, Walsh LJ, et al. The pathogenesis of oral lichen planus. *Crit Rev Oral Biol Med* 2002; **13**: 350–65.
2. Zhao ZZ, Savage NW, Sugerman PB, Walsh LJ. Mast cell/T cell interactions in oral lichen planus. *J Oral Pathol Med* 2002; **31**: 189–95.
3. Khan A, Farah CS, Savage NM, Walsh LJ, Harbrow DJ, Sugerman PB. Th1 cytokines in oral lichen planus. *J Oral Pathol Med* 2003; **32**: 77–83.
4. Hirota J, Osaki T, Tatemoto Y. Immunohistochemical staining of infiltrates in oral lichen planus. *Path Res Pract* 1990; **186**: 625–32.
5. Yamamoto T, Yoneda K, Ueta E, Osaki T. Cellular immunosuppression in oral lichen planus. *J Oral Pathol Med* 1990; **19**: 464–70.
6. Lin SC, Sun A, Wu YC, Chiang CP. Presence of anti-basal cell antibodies in oral lichen planus. *J Am Acad Dermatol* 1992; **26**: 943–7.
7. Sun A, Chiang CP, Chiou PS, Wang JT, Liu BY, Wu YC. Immunomodulation by levamisole in patients with recurrent aphthous ulcers or oral lichen planus. *J Oral Pathol Med* 1994; **23**: 172–7.

8. Lundstrom IM. Serum immunoglobulins and autoantibodies in patients with oral lichen planus. *Int J Oral Surg* 1985; **14**: 259–68.
9. Lodi G, Olsen I, Piattelli A, D'Amico E, Artese L, Porter SR. Antibodies to epithelial components in oral lichen planus (OLP) associated with hepatitis C virus (HCV) infection. *J Oral Pathol Med* 1997; **26**: 36–9.
10. Carrozzo M, Gandolfo S, Lodi G, et al. Oral lichen planus in patients infected or noninfected with hepatitis C virus: the role of autoimmunity. *J Oral Pathol Med* 1999; **28**: 16–9.
11. Lukac J, Brozovi S, Vucicevi-Boras V, Mravak-Stipeti M, Malenica B, Kusi Z. Serum autoantibodies to desmogleins 1 and 3 in patients with oral lichen planus. *Croatian Med J* 2006; **47**: 53–8.
12. Sun A, Chia JS, Chang YF, Chiang CP. Serum interleukin-6 level is a useful marker in evaluating therapeutic effects of levamisole and Chinese medicinal herbs on patients with oral lichen planus. *J Oral Pathol Med* 2002; **31**: 196–203.
13. Chiang CP, Hsieh RP, Chen THH, et al. High incidence of autoantibodies in Taiwanese patients with oral submucous fibrosis. *J Oral Pathol Med* 2002; **31**: 402–9.
14. Jonas G, Pelzer C, Beckert C, Hausmann M, Kapprell HP. Performance characteristics of the ARCHITECT® Anti-HCV assay. *J Clin Virol* 2005; **34**: 97–103.
15. Echevarria JM, Avellon A, Jonas G, Hausmann M, Vockel A, Kapprell HP. Sensitivity of a modified version of the ARCHITECT® Anti-HCV test in detecting samples with immunoblot-confirmed, low-level antibody to hepatitis C virus. *J Clin Virol* 2006; **35**: 368–72.
16. Scully C, El-Kom M. Lichen planus: review and update on pathogenesis. *J Oral Pathol* 1985; **14**: 431–58.
17. Santoro A, Majorana A, Roversi L, et al. Recruitment of dendritic cells in oral lichen planus. *J Pathol* 2005; **205**: 426–34.
18. Lin SC, Sun A. HLA-DR and DQ antigens in Chinese patients with oral lichen planus. *J Oral Pathol* 1990; **19**: 298–300.
19. Sun A, Wu YC, Wang JT, Liu BY, Chiang CP. Association of HLA-Te22 antigen with anti-nuclear antibodies in Chinese patients with erosive lichen planus. *Proc Natl Sci Council ROC(B)* 2000; **24**: 63–9.
20. Hawkins BR, Ma JT, Lam KS, Wang CC, Yeung RT. Association of HLA antigens with thyrotoxic Graves' disease and periodic paralysis in Hong Kong Chinese. *Clin Endocrinol* 1985; **23**: 245–52.
21. Hawkins BR, Lam KS, Ma JT, Wang C, Yeung RT. Strong association between HLA DRw9 and Hashimoto's thyroiditis in Southern Chinese. *Acta Endocrinol* 1987; **114**: 543–6.
22. Chiu HC, Hsieh RP, Hsieh KH, Hung TP. Association of HLA-DRw9 with myasthenia gravis in Chinese. *J Immunogenetics* 1987; **14**: 203–7.
23. Hawkins BR, Lam KS, Ma JT, et al. Strong association between HLA DR3/DRw9 heterozygosity with early-onset insulin-dependent diabetes mellitus in Chinese. *Diabetes* 1987; **36**: 1297–300.

Acknowledgement

This study was supported by a research grant of NSC 95-2314-B002-202 from the National Science Council, Taipei, Taiwan.