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Original Article

Modulation of Serum Antinuclear Antibody Levels by Levamisole Treatment in Patients With Oral Lichen Planus

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Background/Purpose: Serum autoantibodies, including antinuclear antibodies (ANAs), have been found in patients with oral lichen planus (OLP). This study evaluated whether Taiwanese OLP patients had significantly higher frequencies of serum ANAs than healthy control subjects, and whether levamisole treatment could modulate the antibody levels.

Methods: This study used an indirect immunofluorescence technique to measure the baseline serum levels of ANA in a group of 583 Taiwanese OLP patients and 53 healthy control subjects. Seventy-nine ANA-positive OLP patients were treated with levamisole under a regular follow-up schedule in our dental clinic, and their serum ANA levels were measured after treatment.

Results: We found that the frequencies of serum ANA in patients with OLP (23.2%), erosive OLP (EOLP, 23.8%), major EOLP (31.5%), and minor EOLP (18.1%) were all significantly higher than that (5.7%) in healthy control subjects. In addition, major EOLP patients had a significantly higher serum ANA-positive rate than minor EOLP or non-erosive OLP patients. Of 135 ANA-positive OLP patients, 79 were treated with levamisole under a regular follow-up schedule. We found that treatment with levamisole for a period of 2–38 months (mean, 12 ± 9 months) effectively reduced the high mean serum ANA titer (557 ± 98) at baseline to an undetectable level (0) in all ANA-positive OLP patients, regardless of different high initial serum titers of ANA.

Conclusion: There was a significantly higher frequency of serum ANA (23.2%) in Taiwanese OLP patients than in healthy control subjects. Treatment with levamisole for 2–38 months reduced the high serum ANA to an undetectable level, and significantly improved the signs and symptoms in all treated OLP patients.

Key Words: antinuclear antibody, levamisole, oral lichen planus

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Oral lichen planus (OLP) is a chronic inflammatory oral mucosal disease frequently found in the dental clinic. Both antigen-specific and non-specific mechanisms are involved 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 marked subepithelial infiltration of mononuclear cells that are predominantly $CD8^+$. $CD4^+$ cells are observed mainly in the deep lamina propria.[3] An increase in histocompatibility leukocyte antigen (HLA)-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.

Levamisole is an immunomodulator that can modulate T-cell-mediated immunity.[6–9] Our previous studies have found that levamisole can also modulate abnormal serum interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α, anti-basal cell antibody (anti-BCA), and antinuclear antibody (ANA) levels to normal in patients with OLP or erosive OLP (EOLP).[6–9]

The presence of serum autoantibodies including anti-epithelial cell, ANA, smooth muscle antibody (SMA), antimitochondrial antibody (AMA), gastric parietal cell antibody (GPCA), thyroglobulin antibody (TGA), thyroid microsomal antibody (TMA), and anti-desmogleins 1 and 3 antibodies has been shown in several different groups of OLP patients.[9–15]

In this study, we examined the frequencies of serum ANA in a group of 583 Taiwanese OLP patients and in 53 healthy control subjects. We tried to establish whether there was a significantly higher frequency of serum ANA in Taiwanese OLP patients than in healthy control subjects, and whether levamisole treatment could modulate serum ANA levels.

Patients and Methods

Patients and control subjects

The study group consisted of 583 OLP patients (102 men and 481 women; age range, 22–88 years; mean age, 55.0 years) without LP of other mucosal or skin surfaces. The normal control group consisted of 53 healthy subjects (8 men and 45 women; age range, 21–83 years; mean age, 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 February 2010. OLP patients with areca quid chewing habit, hypertension, and autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, pemphigus vulgaris, and cicatricial pemphigoid were excluded. In addition, none of them had taken any prescription medication for at least 3 months before entering the study. The 583 OLP patients included 512 with EOLP (93 men and 419 women; age range, 22–88 years; mean age, 55.3 years) and 71 with non-erosive OLP (NEOLP) (9 men and 62 women; age range, 26–83 years; mean age, 52.9 years). They were selected according to the following criteria: (1) a typical clinical presentation of radiating grayish-white Wickham striae, papules and plaques, separately or in combination (NEOLP), and erosion or ulceration of the oral mucosa (EOLP); and (2) 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. EOLP was further divided into major and minor types according to previously described criteria.[6–8]

All OLP patients, regardless of their subtype, were treated with levamisole that was administered at a dose of 50 mg twice daily for patients with 30–50 kg body weight, or 50 mg three times daily for patients with 50–70 kg body weight, for three consecutive days at the beginning of each two-week interval. Compliance was monitored by asking the patients to record the time at which each drug was taken. Before the start of therapy, clinical data on all cases were recorded according to a set protocol, and the patients were examined by the same dentist during each visit. Patients were monitored once monthly to record clinical responses after treatment.

Blood samples were taken from OLP patients before treatment and from normal control subjects. To assess whether the serum ANA levels in OLP patients were reduced after treatment with levamisole, blood samples were obtained serially every 2–3 months until the serum ANA levels became undetectable. Informed consent was obtained from each patient or control subject before blood sample collection. This study was reviewed and approved by the Human Investigation Review Committee at the National Taiwan University Hospital.

Determination of serum ANA levels

Circulating ANA levels were detected by the indirect immunofluorescence technique with Hep-2 cells (Medical & Biological Laboratories Co., Nagaya, Japan) as substrates, as described previously.[13,15] Hep-2 cells on slides were reacted with serially diluted OLP patients' and control subjects' sera, in a moist chamber at room temperature for 30 minutes. The initial dilution of the patients' and control subjects' sera was 1:20 with phosphate-buffered saline (PBS). After washing with PBS, the sections were incubated with fluorescein-isothiocyanate-labeled, goat anti-human IgG antiserum (Boehringer Mannheim Biochemicals, Indianapolis, IN, USA), which had been prediluted and kept in a dropper vial by the manufacturer, and was ready-to-use for another 30 minutes. The sections were washed again, mounted with buffered glycerine, and examined with an Olympus fluorescence microscope (Tokyo, Japan). Sera were scored as positive for ANA when they produced fluorescence at a dilution of 160-fold or more.

Statistical analysis

The difference in frequency of serum ANA was compared between any two groups by the χ^2 test. The serum levels of ANA at baseline and after treatment were compared to each other using a paired *t* test. The result was considered to be significant if the *p* value was < 0.05.

Results

One hundred and thirty-five OLP patients (23.2%) had an ANA titer $\geq$ 160-fold in their sera. The frequencies of serum ANA in different groups of OLP patients and 53 healthy control subjects are shown in Table 1. We found that the frequencies of serum ANA in patients with OLP (23.2%, $p = 0.005$), EOLP (23.8%, $p = 0.004$), major EOLP (31.5%, $p = 0.000$), and minor EOLP (18.1%, $p = 0.040$) were all significantly higher than that (5.7%) in healthy control subjects. In addition, OLP patients > 50 years of age and male or female

Table 1. Frequencies of serum antinuclear antibody in different groups of oral lichen planus patients and 53 healthy control subjects

Groups	ANA positive patient number (%)	*p*
OLP ($n = 583$)	135 (23.2)	0.005[a]
≤ 50 yr ($n = 185$)	31 (16.8)	0.070[a]
> 50 yr ($n = 398$)	104 (26.1)	0.002[a]
Male ($n = 102$)	21 (20.6)	0.028[a]
Female ($n = 481$)	114 (23.7)	0.005[a]
EOLP ($n = 512$)	122 (23.8)	0.004[a]
Major type ($n = 219$)	69 (31.5)	0.000[a]
Minor type ($n = 293$)	53 (18.1)	0.040[a]
NEOLP ($n = 71$)	13 (18.3)	0.071[a]
Healthy controls ($n = 53$)	3 (5.7)	

[a]*Comparison between healthy control and any other groups by χ^2 test. ANA = antinuclear antibody; EOLP = erosive oral lichen planus; NEOLP = non-erosive oral lichen planus.*

Table 2. Comparison of serum antinuclear antibody positivity between different groups of oral lichen planus patients

	ANA (+) ($n=135$)	ANA (–) ($n=448$)	p (χ^2 test)
Age (yr)			0.017
≤ 50 ($n=185$)	31	154	
> 50 ($n=398$)	104	294	
Sex			0.584
Male ($n=102$)	21	81	
Female ($n=481$)	114	367	
OLP type			0.377
EOLP ($n=512$)	122	390	
NEOLP ($n=71$)	13	58	
EOLP type			0.000
Major ($n=219$)	69	150	
Minor ($n=293$)	53	240	
OLP type			0.046
Major EOLP ($n=219$)	69	150	
NEOLP ($n=71$)	13	58	

ANA = antinuclear antibody; EOLP = erosive oral lichen planus; NEOLP = non-erosive oral lichen planus.

patients also had a significant higher frequency of serum ANA than healthy control subjects had (Table 1). Moreover, OLP patients > 50 years of age had a significantly higher frequency of serum ANA (26.1%) than those aged ≤ 50 years (16.8%, $p=0.017$, Table 2). In addition, major EOLP patients had a significantly higher frequency of serum ANA (31.5%) than minor EOLP (18.1%, $p=0.000$) or NEOLP (18.3%, $p=0.046$, Table 2) patients had.

Of 135 ANA-positive OLP patients, 79 were treated with levamisole under a regular follow-up schedule in our dental clinic. We found that treatment with levamisole for a period of 2–38 months (mean, 12 ± 9 months) effectively reduced the high mean serum ANA level (557 ± 98) at baseline to an undetectable level (0) in all 79 ANA-positive OLP patients, including those with EOLP, major or minor EOLP, and NEOLP (Table 3). Furthermore, when OLP patients were divided into five different groups according to different initial serum ANA titers that ranged from 1:40 to 1:5120, all the high serum ANA levels at baseline were effectively reduced to an undetectable level (0, all p values = 0.000) after treatment with levamisole for 2–38 months (Table 3). Moreover, OLP patients with higher ANA titers usually required longer treatment duration to reduce ANA titer to an undetectable level than did those with lower ANA titers (Table 3). The 79 ANA-positive OLP patients also had significant improvement in signs and symptoms of OLP after levamisole treatment for 2–38 months (such as a reduction in lesion size and pain caused by the lesion, healing of erosive or ulcerative lesions, and transformation of EOLP into NEOLP).

Discussion

This study showed a serum ANA-positive rate of 23.2% in 583 OLP patients. Our previous study demonstrated the presence of serum ANA in 28.1% of 320 OLP patients.[15] We suggest that the difference in the serum ANA-positive rate between these two groups of OLP patients was due to different sample sizes in these two studies. In the present study, we treated the OLP patients with levamisole and found that serum ANA was reduced to an undetectable level after treatment for 2–38 months, regardless of different high initial serum titers of ANA. Our previous studies also have shown the disappearance of serum anti-BCA in three of six anti-BCA-positive EOLP patients and the disappearance of serum ANA in three ANA-positive EOLP patients after levamisole treatment.[9] In addition, treatment with levamisole for 0.5–7.5 months can significantly reduce the abnormally high serum IL-6, IL-8 and TNF-α levels to normal in patients with OLP or EOLP.[7,8] The most important factor is that the reduction of autoantibody or cytokine levels after levamisole treatment is always accompanied by significant improvement of signs and symptoms in OLP patients.[6–9] These findings suggest that levamisole treatment shifts the abnormal serum autoantibody and cytokine levels to normal in OLP patients, and is an effective treatment modality for OLP.

Table 3. Serum titers of antinuclear antibody before and after treatment with levamisole in 79 oral lichen planus patients, including 36 with major and 35 with minor erosive oral lichen planus, and eight with non-erosive oral lichen planus

Treatment modality	Patient type or ANA titer	Duration of treatment (mo)		Serum titers of ANA (Fold of dilution of serum)		p^a
		Range	Mean ± SD	At baseline	After treatment	
				Mean ± SEM	Mean ± SEM	
Levamisole	OLP ($n = 79$)	2–38	12 ± 9	557 ± 98	0	0.000
Levamisole	EOLP ($n = 71$)	2–38	12 ± 9	578 ± 108	0	0.000
Levamisole	Major type ($n = 36$)	2–38	14 ± 10	828 ± 183	0	0.000
Levamisole	Minor type ($n = 35$)	2–38	9 ± 8	321 ± 98	0	0.002
Levamisole	NEOLP ($n = 8$)	4–29	14 ± 8	370 ± 147	0	0.040
Levamisole	ANA titer ≥ 1280 ($n = 11$)	13–38	24 ± 9	2444 ± 321	0	0.000
Levamisole	ANA titer = 640 ($n = 11$)	8–32	19 ± 8	640	0	0.000
Levamisole	ANA titer = 320 ($n = 23$)	5–29	13 ± 7	320	0	0.000
Levamisole	ANA titer = 160 ($n = 8$)	2–15	6 ± 5	160	0	0.000
Levamisole	ANA titer ≤ 80 ($n = 26$)	2–12	5 ± 2	55 ± 4	0	0.000

[a]Comparison of serum ANA titers between patients at baseline and after treatment by paired t test. ANA = antinuclear antibody; SD = standard deviation; SEM = standard error of the mean; OLP = oral lichen planus; EOLP = erosive oral lichen planus; NEOLP = non-erosive oral lichen planus.

Previous studies have demonstrated an overall incidence of 27–82% for serum autoantibodies in several different groups of OLP or EOLP patients.[9–15] Serum anti-BCA was found in 34 (54%) of 63 OLP patients.[10] Serum autoantibodies including rheumatoid factor, ANA and SMA were demonstrated in 27% of 30 OLP patients and in 9% of 23 control subjects.[11] The presence of circulating antibodies to epithelial antigen was shown in 57% of 14 hepatitis C virus antibody (HCVA)-positive OLP patients and in none of 14 HCVA-negative patients.[12] Carrozzo et al[13] discovered the presence of serum autoantibodies, including ANA, SMA, AMA, GPCA, and antithyroid antibody in 41% of 27 HCVA-positive OLP patients and in 52% of 23 HCVA-negative patients. Lukac et al[14] demonstrated by enzyme-linked immunosorbent assay significantly higher concentrations of circulating autoantibodies to desmogleins 1 and 3 in 32 EOLP patients compared with 50 healthy controls. Indirect immunofluorescence also revealed significantly higher frequencies of desmoglein autoantibodies in EOLP patients (82%, 18/22) than in healthy controls (5%, 1/20). Our recent study showed an overall incidence of 60.9% for the presence of serum autoantibodies in a large group of 320 OLP patients, and significantly higher frequencies of serum ANA, GPCA, TGA and TMA in OLP patients than in healthy control subjects.[15] The above findings suggest that there are significantly higher frequencies of serum autoantibodies in OLP patients than in healthy control subjects.

The present study showed a significantly higher serum ANA positive rate in major EOLP than in minor EOLP or NEOLP patients. Major EOLP lesions caused greater destruction of oral mucosa and therefore might have released more nuclear antigens into the local tissues and blood circulation than did minor EOLP or NEOLP lesions. These nuclear antigens could have been phagocytosed and processed by macrophages and B cells, and in turn, presented to helper/inducer T cells in the oral mucosal lesions, regional lymph nodes, and blood circulation of OLP patients. With the help of T cells, antigen-specific activated B cells thus produced high levels of ANA in the local oral mucosal lesions and blood circulation of OLP patients. In addition, our recent study also has shown that HCVA positivity and

TGA/TMA positivity are another two risk factors that contribute to the presence of serum ANA in OLP patients.[15] HCVA-positive and TGA/TMA-positive OLP patients may also have underlying chronic liver and autoimmune thyroid diseases, respectively. Therefore, they can have more nuclear antigens released in the local liver and thyroid tissues and blood circulation. By similar mechanisms, these released nuclear antigens may stimulate the antigen-specific activated B cells to produce more ANA. The locally produced autoantibodies in the interstitial fluid may diffuse into blood capillaries or be drained into the lymphatic vessels, and finally reach the blood circulation. The locally secreted ANA, together with that produced in the blood circulation eventually give rise to significantly higher levels of serum ANA in OLP patients than in healthy control subjects. The disappearance of the serum anti-BCA antibodies and ANA after treatment-induced healing of the OLP lesions in previous studies[9,10] and in the present study also suggests that the principal autoantigens come from the local OLP lesions.

In conclusion, there was a significantly higher frequency of serum ANA (23.2%) in Taiwanese OLP patients than in healthy control subjects. Treatment with levamisole for 2–38 reduced the high serum ANA concentrations to undetectable levels, and significantly improved signs and symptoms of OLP patients. Therefore, we conclude that levamisole treatment can modulate the high serum ANA titer to undetectable levels in OLP patients, and is an effective treatment modality for OLP.

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