

# The Impact of Immunotherapeutic G2 Vaccine on Treatment of Oral Lichen Planus

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## KEY WORDS

G2 vaccine;  
Immunotherapeutic;  
Oral lichen planus.

## ABSTRACT

**Statement of Problem:** Oral lichen planus (OLP) is a cell-mediated immune condition for which several topical and systemic treatments are available.

**Purpose:** The purpose of this study was to evaluate the efficacy of G2 vaccine on treatment of OLP.

**Materials and Methods:** Ten OLP patients (7 females, 3 males) were enrolled in this study. Diagnosis of OLP was based on the clinical criteria and histological study. The patients received subcutaneous injection of 0.4 cc of G2 vaccine, once a week for twelve weeks. Oral symptoms before and after treatment were assessed by visual analogue scale. The data were analyzed, using student's t-test.

**Results:** In this study, improvement in the lesion size was observed in the majority of the patients (good for 3 and poor for 5) while two patients showed no changes and no complete response was observed. The patients' symptoms (pain/burning) decreased significantly ( $p = 0.01$ ). The CD4+ count and CD4+/CD8+ ratio was increased after therapy.

**Conclusion:** The present study indicated that the patients' symptoms were decreased simultaneously with the increase in CD4+ counts and rise in CD4+/CD8+ ratio after inoculation of G2 vaccine. The findings suggest further studies with more sample size and also assessment of more types of cytokines for evaluation of the efficacy of G2 vaccine.

Received Nov. 2010;  
Received in revised form Dec. 2010;  
Accepted Jan. 2011

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## Introduction

Oral lichen planus (OLP) is a chronic inflammatory disease and one of the common oral

mucosal diseases in patients referring to dental clinics [1]. The reported prevalence rates of OLP vary from 0.5% to 2.2% of the population. The

typical age of presentation is between 30 and 60 years, and it is more frequently seen in women [2]. The etiologic factor of OLP is poorly understood. There is extensive evidence to suggest that cellular immune system dysfunction plays a key role in the onset and perpetuation of the disorder [3-5]. OLP is a cell-mediated immune condition that may be regulated by various cytokines. Several topical and systemic treatments are available for patients with OLP but therapeutic responsiveness may differ among patients [6-8].

Immunotherapeutic G2 vaccine (IG2V) is a derivative lipid from the buffalo spleen without any side effects increasing T helper lymphocytes ( $CD_4^+$ ) response. This results in activation of immune T helper-1 ( $Th_1$ ) response and suppression of T helper-2 ( $Th_2$ ) immune response. Evidence from mouse and human models' investigations has indicated that T helper cell can be classified into at least two functional subsets,  $Th_1$  and  $Th_2$ , on the basis of their cytokine profiles.  $Th_1$  cells are characterized by the production of IL2, IFN $\gamma$  and are critical in the cell mediated immunity, but  $Th_2$  cells are characterized by the production of IL4, IL5, IL6 and IL13 play important roles in humoral immunity [9]. Some studies have observed elevated IL6 levels in patients with OLP and oral squamous cell carcinoma (OSCC) compared with that in normal subjects, reflecting the importance of this cytokine [10-13]. Further reports have indicated a decrease in helper lymphocytes ( $CD_4^+$ ) and ( $CD_4^+$ )/( $CD_8^+$ ) ratio in patients with reticular, atrophic /erosive and plaque-like OLP [14-16]. During these years more than a thousand of different allergic, asthmatic patients have used G2 vaccine without any important side effects (which are not published yet). Topical and systemic corticosteroid therapy result in reducing inflammation, sign and symptoms of OLP but usually lesions will be relapsed. So use of drugs is considered for adjustment and regul-

ation of immune system.

This study investigated the effects of G2 vaccine on the peripheral blood lymphocytes ( $CD_4^+$ ,  $CD_8^+$  and their ratio) and its possible Immunotherapeutic effect on OLP.

### Materials and Methods

The present study was an interventional clinical-trial study carried out in Oral Medicine Department of Tehran University of Medical Sciences. According to a pilot study, ten patients (3 male, 7 female) were enrolled in this study with atrophic erosive OLP based on the clinical criteria confirmed by histological study of the biopsy specimen of the lesion. The mean age of the patients was 42.9 years (range 27-68). Patients with systemic diseases, pregnant and breastfeeding women and those with a history of taking corticosteroids and/or routine medicine in the previous month were excluded from the study.

G2 vaccine has been prepared from buffalo spleen lipid and registered as a patent in Iranian Patent Office as immune system activator vaccine (innovation register no: 36679, 28<sup>th</sup> of October 2006). G2 vaccines have been studied on several animal models of asthma, breast and colorectal cancers successfully without any serious side effects [17, 18] and have been approved by the ethical committee of Tehran University of Medical Sciences to be used in clinical trials on asthmatic patients. Informed consents were obtained from all volunteer patients.

Briefly, the spleen was crashed in small pieces and diluted in alcohol for a few days. Then, they were centrifuged at 800\*g for 30 minutes and the supernatants were dried to get 20 microgram per milliliter of concentrate. G2 vaccine components include: different kinds of lipids, glucose, cholesterol, and triglyceride as liposome and water soluble materials. 0.4 cc (containing 8 microgram of effective substance) of G2

vaccine was injected subcutaneously in the upper deltoid muscle region, once a week for a period of twelve weeks.

The lesion size and severity of pain were assessed by scaled tongue blade and visual analogue scale (VAS), respectively. Before the first and one week after the last injections, assessment of CBC (Diff), (CD<sub>4</sub><sup>+</sup>), (CD<sub>8</sub><sup>+</sup>) and (CD<sub>4</sub><sup>+</sup>)/(CD<sub>8</sub><sup>+</sup>) ratio was performed by floctometry. The clinical data were scored according to the criteria used by Thongprasom et al [19] (Table 1). Response to treatment was graded as complete when the scores, according to Thongprasom et al, were either 0 or 1, good when scores decreased by >50% from the baseline, poor when scores decreased by <50% from the baseline and as no response when the lesions were unchanged.

**Table 1** Clinical criteria for assessment of OLP lesions

Scores	Clinical characteristic
0	No lesion
1	Mild white striae, no erythematose area
2	White striae with atrophic area<1cm <sup>2</sup>
3	White striae with atrophic area>1cm <sup>2</sup>
4	White striae with erosive area<1cm <sup>2</sup>
5	White striae with erosive area>1cm <sup>2</sup> ,or ulcerative lesions

The pain or/and burning sensation was scored by visual analogue scale [20].

The size of the lesions and intensity of pain were recorded weekly. The patients were explained not to consume any local and systemic medication during the research. Statistical analysis was performed, using SPSS version 13 software. Paired sample t-test was used for assessing the change in symptoms (pain and burning sensation), size of the lesion and (CD<sub>4</sub><sup>+</sup>)/(CD<sub>8</sub><sup>+</sup>) ratio.

## Results

Ten adult patients (7 females, 3 men with a mean age of 42.9) were enrolled in the study. The most common site of involvement in our study

was the buccal mucosa followed by the gingival tissue. Regarding clinical signs one week after the last injections, three patients had good response. Improvement of lesions by a decrease of the clinical scores by <50% (poor response) was noted in five patients. No change of the lesion after the weeks of injections was observed in two patients. The mean VAS pain score was 4.6 before the injections and it decreased to 3.2 one week after the last injections, being statistically significant ( $p = 0.01$ ).

The mean counts of (CD<sub>4</sub><sup>+</sup>), (CD<sub>8</sub><sup>+</sup>) and (CD<sub>4</sub><sup>+</sup>)/(CD<sub>8</sub><sup>+</sup>) ratio before injections were 40.94, 30.27 and 1.46, respectively. The (CD<sub>4</sub><sup>+</sup>) counts and (CD<sub>4</sub><sup>+</sup>)/(CD<sub>8</sub><sup>+</sup>) ratio increased at the end of the injections and they were statistically significant ( $p = 0.04$ ) (Table 2). The (CD<sub>8</sub><sup>+</sup>) counts decreased at the end of the injections but were not statistically significant. No serious side effects of G2 vaccine were recorded.

**Table 2** Assessment of CD4+, CD8+ and CD4+/CD8+ ratio before and after G2 vaccine injections

Patients	Before injections			After injections		
	CD4+	CD8+	CD4+/CD8+	CD4+	CD8+	CD4+/CD8+
1	40.6	21.2	1.91	46.6	25.2	1.84
2	44.4	33.2	1.33	41.6	35.4	1.17
3	28.1	28.7	0.97	37.5	31.1	1.20
4	34.2	38.9	0.87	62.3	31.2	1.99
5	33.6	32.6	1.03	43.8	26.5	1.65
6	37.5	33.2	1.12	62.0	33.1	1.87
7	38.7	38.1	1.01	48.9	27.3	1.79
8	59.2	37.8	1.56	50.6	28.3	1.78
9	45.0	20.0	2.25	52.7	29.2	1.80
10	48.4	19.0	2.54	33.7	17.1	1.97
Mean	40.97	30.27	1.46	47.97	28.44	1.71

## Discussion

OLP is a chronic immunological disease which has no definite cure at present [21]. The Th<sub>1</sub>:Th<sub>2</sub> balance by hypothesis emerged in the late 1980. Currently, much of the literature has elevated the Th<sub>1</sub>:Th<sub>2</sub> balance concept to the level of paradigm; moreover, cytokine and anti-cytokine therapies, derived from the concept of modulat-

ing Th<sub>1</sub>: Th<sub>2</sub> balance, have been successfully applied to several auto-immune diseases including sjogren syndrome [22, 23].

Al-fouzan et al [15] have found that the proportions of both total (CD<sub>3</sub><sup>+</sup>) T cells and helper (CD<sub>4</sub><sup>+</sup>) T cells were significantly lower in patients with lichen planus than in healthy controls. Their results also indicated that this reduction increased slightly over time.

This preliminary clinical trial evaluated the efficacy of immune therapeutic G2 vaccine in treatment of OLP. Early immunologic studies of patients with lichen planus indicated reduced (CD<sub>8</sub><sup>+</sup>) lymphocyte counts in the peripheral blood, suggesting altered suppressor function [22-25]. Other authors have reported an imbalance between (CD<sub>4</sub><sup>+</sup>) and (CD<sub>8</sub><sup>+</sup>) T lymphocyte population in OLP patients [26].

In our study, the lesion size improvement was observed in the majority of the patients (good for 3 and poor for 5) while two patients showed no changes and no complete response was observed in the subjects. The patients' symptoms (pain /burning) decreased significantly. The patients' (CD<sub>4</sub><sup>+</sup>) counts and (CD<sub>4</sub><sup>+</sup>)/(CD<sub>8</sub><sup>+</sup>) ratio were lower than normal before injections. The (CD<sub>4</sub><sup>+</sup>) count and (CD<sub>4</sub><sup>+</sup>)/(CD<sub>8</sub><sup>+</sup>) ratio were increased after therapy. This increase may be due to the effect of G2 vaccine and possibly justifies T helper activating effect of G2 vaccine.

Also, Carrozzo et al [27] have performed a very comprehensive study of T cell subsets in the peripheral blood of the patients with OLP. Their results are in many respects consistent with those of this study and also in accordance with those of Yamamoto et al [28] in that reduction in (CD<sub>4</sub><sup>+</sup>)/(CD<sub>8</sub><sup>+</sup>) ratio was shown. In partial accordance with the findings of Sugerman et al [16, 29], these authors suggest that OLP involves migration of T cells from the blood stream to the oral mucosa, resulting in alterations in ly-

mphocyte subsets in the peripheral blood.

But, Zhou G et al [30] have found that Th<sub>2</sub> immune response is predominant in OLP and dexamethasone is an immune suppressant inhibitor of Th<sub>1</sub>, Th<sub>2</sub> cytokines which is different from the results of our study. Furthermore, Gao man et al [31] showed higher serum and oral IL6 level in patients with ulcerative OLP. This confirms our finding which has shown the Th<sub>2</sub> response in OLP. This could be due to local and systematic production of IL6 by many cell types. Moreover, Yamamoto T et al [32] detected an increase in IL4 and TNF $\alpha$  in the serum from one third of OLP patients and IL6 in all patients. This is a little bit different from our finding. Also, Dorota et al [33] have reported that OLP patients' neopterin expression level and (CD<sub>4</sub><sup>+</sup>), (CD<sub>16</sub><sup>+</sup>), (CD<sub>8</sub><sup>+</sup>), and CD<sub>45</sub> RA antigens on T lymphocytes significantly differed from the controls. Also, he has mentioned that the level of IL18 was higher in patients significantly as compared with healthy controls.

### Conclusion

The present study indicated that OLP patients' symptoms were decreased simultaneously with the increase in (CD<sub>4</sub><sup>+</sup>) counts and rise in (CD<sub>4</sub><sup>+</sup>)/(CD<sub>8</sub><sup>+</sup>) ratio after inoculation of G2 vaccine. This preliminary finding may help to find more information of pathogenesis of OLP and lead to their treatment. However, further studies with more sample size and also assessment of more types of cytokines for evaluation of the efficacy and mechanisms of G2 vaccine are suggested.

### Acknowledgments

This study was financially supported by Dental Research Center of Tehran University of Medical sciences. The authors would like to thank Dr. Mohaghegh Hazrati and Foundation Researcher on Industrial Biotechnology for providing us with

the registry IRCT Number 2950.

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