"Tien-Hsien" liquid modulates antigen-stimulated cytokine production by T-cells from patients with erosive oral lichen planus

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Erosive oral lichen planus (EOLP) is a T-cell-mediated inflammatory oral mucosal disease. Tien-Hsien liquid (THL) is an extract of Chinese medicinal herbs that can modulate the antigen-stimulated proliferative response of and cytokine production by T cells from patients with recurrent aphthous ulcerations. In this study, we tested whether THL could modulate the antigen-stimulated cytokine production by T cells from EOLP patients (EOLP-T cells). T cells isolated from 15 EOLP patients were incubated with phytohemagglutinin (PHA), glutaraldehyde-inactivated tetanus toxoid (TT), glucosyltransferase D (GtfD), or antigens of Streptococcus mutans in the presence or absence of THL. Levels of interleukin (IL)-2, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, IL-6, and IL-10 in the supernatants of T-cell cultures were measured by cytokine enzyme-linked immunosorbent assay (ELISA) kits. We found that THL significantly increased PHA- and TT-stimulated TNF-α and IL-6 production by EOLP-T cells. However, THL also significantly decreased GtfD-stimulated IL-10 production and S. mutans-stimulated TNF-α and IL-10 production by EOLP-T cells. Because THL both increased and decreased antigen-stimulated cytokine production by EOLP-T cells, we concluded that THL modulates antigen-stimulated cytokine production by EOLP-T cells. ***(J Dent Sci, 3(3) : 159-166 , 2008)***

Key words: **Chinese medicinal herbs, Tien-Hsien liquid, T lymphocytes, erosive oral lichen planus.**

Oral lichen planus (OLP) is a chronic inflammatory oral mucosal disease. Although the pathogenesis of OLP is still unclear, both antigen-specific and non-specific mechanisms may be involved. Antigen-specific mechanisms in OLP 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, predominantly $CD8^+$ cells. $CD4^+$ cells are mainly found in the deep lamina propria[3]. Increases in histocompatibility leukocyte antigen (HLA)-DR-positive $CD3^+$ cells in both local lesional tissues and peripheral lymphocytes also indicate T-cell activation in OLP[4,5]. Those findings suggest that OLP is a T-cell-mediated inflammatory disease.

Our previous study found the disappearance of serum anti-nuclear antibody (ANA) in 3 patients with erosive OLP (EOLP) and the disappearance of serum

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anti-basal cell antibodies (anti-BCAs) in 50% (3/6) of anti-BCA-positive EOLP patients after levamisole treatment[6]. Moreover, we observed that both major- and minor-type EOLP patients can obtain a significant reduction of abnormally high serum squamous cell carcinoma-associated antigen (SCCA) levels after treatment with levamisole and/or Chinese medicinal herbs (a water extract of *Radix astragale*, *Fructus lycii*, and *Fructus ziziphi jujubae*)[7]. In addition, we found that levamisole treatment significantly reduced abnormally high serum interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α levels to normal levels in OLP patients[8-10]. These findings suggest that treatment with an immunomodulator (levamisole) for several months can reverse abnormally high levels of autoantibodies of SCCA, IL-6, IL-8 or TNF-α to normal levels.

IL-2 is mainly secreted by activated T cells. It induces T-cell proliferation, potentiates B-cell growth, and enhances natural killer (NK) cell and monocyte activation[11]. Interferon (IFN)-γ is a pleotropic cytokine that plays an essential role in both the innate and adaptive phases of the immune response. NK, $CD8^+$, and $CD4^+$ Th1 cells are the most potent sources of IFN-γ[12]. TNF-α is a proinflammatory cytokine which is secreted by activated monocytes, macrophages, and many other cells including B cells, T cells, mast cells, and fibroblasts[13,14]. TNF-α has stimulatory activities on activated T cells. It also induces the secretion of IL-1, IFN-γ, and IL-6[15].

IL-6 is a multifunctional cytokine that participates in inflammatory and immune responses. IL-6 is produced by activated monocytes, macrophages, endothelial cells, fibroblasts, keratinocytes, and activated T and B cells in response to induction by a variety of stimuli which include other cytokines[16]. Its immunological activities include B-cell differentiation and stimulation of immunoglobulin G (IgG) secretion, T-cell growth and differentiation, and cytotoxic T lymphocyte differentiation[17].

IL-10 is an important immunosuppressive and anti-inflammatory cytokine released by both T cells and antigen-presenting cells[18]. IL-10 can inhibit the activation and effector function of several cell types including T cells, monocytes, and macrophages. IL-10 directly affects the function of Th1 cells by inhibiting the production of a number of cytokines, including IL-2, IFN-γ, and TNF-α[19].

Approximately 1%~2% of tissue-infiltrating mononuclear cells from OLP lesions are positive for IL-2, TNF-α, and IL-10 messenger (m)RNAs, and expressions of IFN-γ and IL-10 mRNAs were found in cultured T lymphocytes from OLP lesions by polymerase chain reaction (PCR)[20]. IFN-γ and IL-6 mRNAs were detected within proliferating $CD3^+$ T lymphocytes in the upper lamina propria and were localized in basal and suprabasal keratinocytes of OLP lesions (OLP-keratinocytes)[21]. Tissue culture studies showed that OLP-keratinocytes, tissue-infiltrating mononuclear cells from OLP lesions, and peripheral blood mononuclear cells from OLP patients (OLP -PBMCs) can produce IL-2, IFN-γ, TNF-α, IL-6, and IL-10[22-24]. In addition, increased serum levels of TNF-α and IL-6 were discovered in OLP patients[8-10,24,25].

Our previous studies found that Tien-Hsien liquid (THL, Feida Union Pharmaceutical Manufactory, El Monte, CA, USA), an extract of Chinese medicinal herbs, can modulate the antigen-stimulated proliferative response of T cells[26] and cytokine production by T cells from patients with recurrent aphthous ulcerations[27]. Because THL has both immunopotentiation and immunosuppressive effects and can either stimulate or inhibit the lymphoproliferative response and cytokine production by T cells[26-29], it can be used as an immunomodulating agent to restore the altered cellular or humoral immunity in EOLP patients. In this study, we further tested whether THL can modulate the phytohemagglutinin (PHA)-, glutaraldehyde- inactivated tetanus toxoid (TT)-, glucosyltransferase D (GtfD)-, and S. mutans-stimulated cytokine production by EOLP-T cells.

MATERIALS AND METHODS

Subjects

After approval by the Hospital Review Board, 15 EOLP patients (4 men and 11 women, mean age 50 ± 10, range 28~65 years) without LP of other mucosal or skin surfaces were included in this study. All patients were seen consecutively, diagnosed, and treated in the oral mucosal disease clinic of the Dental Department of National Taiwan University Hospital from October 2003 to June 2004. None of them had taken any prescription medication for at least 3 months before entering the study. They were selected based on the following criteria: (1) a typical clinical

presentation of radiating grayish-white Wickham striae and erosion or ulceration of the oral mucosa, and (2) biopsy specimens characteristic of OLP, that is, hyperkeratosis or parakeratosis, a slightly acanthotic epithelium with liquefaction degeneration of basal epithelial cells, and a pronounced band-like lymphocytic infiltrate in the lamina propria.

Stimulation antigens

PHA was purchased from Sigma (Sigma, St. Louis, MO, USA). TT was provided by Ming-Yi Liau of the Department of Health, Center for Disease Control, Vaccine Center, Taipei, Taiwan. Recombinant GtfD was made in our laboratory, and the detailed procedures for production and purification of recombinant GtfD were previously described[30,31]. Streptococcus mutans GS-5 was grown in brain heart infusion broth (Difco, Detroit, MI, USA). These 4 antigens were selected because they stimulated proliferative responses of T cells and cytokine production by T cells in patients with recurrent aphthous ulcerations in our previous studies[26,27]. All antigens used for stimulating cytokine production by EOLP-T cells, including GtfD and the other reagents, exhibited undetectable endotoxin levels (< 30 pg/ml) as determined by the Limulus amebocyte lysate assay (Sigma).

Modulating drugs

THL and active hexose-correlated compound (AHCC) were used to modulate antigen-stimulated cytokine production by EOLP-T cells. Concentrations at a 1:1000 dilution for THL and 5 μg/ml for AHCC were selected because these 2 concentrations had no cytotoxic effects on PBMCs or T cells cultured in vitro and did not stimulate T cells from healthy control subjects after 5 days of incubation in our previous study[26]. The composition as well as the pharmacological and immunological effects of the major ingredients of THL were described in previous studies[26,28,29] AHCC is a proprietary extract prepared from co-cultured mycelia of several species of Basidiomycete mushrooms, including shiitake (*Lentinus edodes*). The extract is made using hot water following an enzyme pretreatment; it contains polysaccharides, amino acids, and minerals, and is orally bioavailable[32]. Animal research and preliminary human studies indicated that AHCC has anticancer efficacy[32]. In addition, AHCC has hepatoprotective and immunopotentiation effects, and can prolong the survival of patients with hepatocellular carcinoma (HCC) after surgical resection[32-34].

Cell preparation and antigen-stimulated cytokine production assay

Peripheral blood samples were collected from 15 EOLP patients after obtaining the patients' informed consent. The preparation of enriched T-cells and irradiated autologous PBMCs from blood samples was described in our previous study[27]. In antigen-stimulated cytokine production assays, enriched T cells (1×10^5 cells/well) were cultured in the presence of irradiated autologous PBMCs (2×10^5 cells/well) in RPMI 1640, supplemented with 2% fetal calf serum, 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol, penicillin (100 μg/ml), streptomycin sulfate (100 μg/ml), and 2% thiophene-2-carboxylic acid hydrazide (Celox Laboratories, Inc., San Diego, CA. USA). To test whether THL and AHCC had modulating effects on cytokine production by T cells, 3 replicates of T-cell culture from 15 EOLP patients were incubated with PHA (1 μg/ml), TT (10 μg/ml), recombinant GtfD (10 μg/ml), or antigens of S. mutans (2×10^5 colony-forming units (CFU)) in the presence or absence of THL (1:1000 dilution) or AHCC (5 μg/ml). Because previous studies showed that AHCC has immunopotentiating effects on the immune system of HCC patients[32-34], in this study, AHCC was used as a positive control agent with potentiating effects on antigen-stimulated cytokine production by EOLP-T-cells. Incubation was performed at 37 °C in a humidified atmosphere with 5% CO_2 for 5 days. Culture supernatants were collected on day 5 and then frozen at -20 °C for future analysis.

Detection of cytokines

Cytokines were quantitated by enzyme-linked immunosorbent assay (ELISA) kits (Quantikine; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instruction as described previously[27,35]. The minimum detectable cytokine concentrations were estimated to be 1 pg/ml for IL-2, IFN-γ, TNF-α, and IL-10 as well as 1.6 pg/ml for IL-6. Cytokine levels are expressed as the mean ± the standard error of the mean (SEM).

Statistical analysis

Mean cytokine levels were compared between the no-antigen and antigen-only groups as well as between the antigen-only and antigen-plus-THL or antigen-plus-AHCC groups by the Wilcoxon signed rank test. Results were considered to be significant at a p value of < 0.05.

RESULTS

In this study, we tested whether THL (1:1000 dilution) or AHCC (5 μg/ml) had modulating effects on PHA- (1 μg/ml), TT- (10 μg/ml), GtfD- (10 μg/ml), and S. mutans-stimulated (2×10^5 CFU) secretions of IL-2, IFN-γ, TNF-α, IL-6, and IL-10 by EOLP-T cells. Compared to the spontaneous release of IL-2 (8 ± 2 pg/ml) by EOLP-T cells, PHA and TT stimulated EOLP-T cells to secrete higher levels of IL-2 (59 ± 10 and 25 ± 9 pg/ml, respectively), but the differences were not significant ($p > 0.05$) (Table 1). THL had no significant modulating effect on antigen-stimulated IL-2 production by EOLP-T cells. However, AHCC significantly reduced PHA-stimulated IL-2 production from 59 ± 10 to 25 ± 5 pg/ml ($p < 0.05$, Table 1).

IFN-γ secretion by EOLP-T cells was elicited to significantly higher levels with stimulation by PHA (72 ± 13 pg/ml, $p < 0.001$), GtfD (130 ± 17 pg/ml, $p < 0.001$), and S. mutans (86 ± 27 pg/ml, $p < 0.01$) compared to the spontaneous release of IFN-γ (3 ± 2 pg/ml) by EOLP-T cells without antigen stimulation. It was obvious that GtfD was the strongest antigen for stimulating IFN-γ secretion by EOLP-T cells, followed by S. mutans and PHA. THL had no significant modulating effect on antigen-stimulated IFN-γ production by EOLP-T cells. However, AHCC significantly enhanced GtfD-stimulated IFN-γ production by EOLP-T cells from 130 ± 17 to 278 ± 39 pg/ml ($p < 0.005$, Table 2).

Significantly higher levels of TNF-α production by EOLP-T cells from 6 ± 3 to 52 ± 9 ($p < 0.001$), 396 ± 34 ($p < 0.001$), and 491 ± 27 pg/ml ($p < 0.001$) were respectively elicited by stimulation with PHA, GtfD, and S. mutans (Table 3). THL significantly augmented PHA- or TT-stimulated TNF-α production by EOLP-T cells from 52 ± 9 to 275 ± 32 pg/ml ($p < 0.001$) or from 9 ± 3 to 253 ± 29 pg/ml ($p < 0.001$), respectively. THL also significantly lowered S. mutans-stimulated TNF-α production by EOLP-T cells from 491 ± 27 to 311 ± 27 pg/ml ($p < 0.001$). AHCC significantly raised PHA- and TT-stimulated TNF-α production by EOLP-T cells from 52 ± 9 to 148 ± 42 pg/ml ($p < 0.05$) and 9 ± 3 to 108 ± 16 pg/ml ($p < 0.001$), respectively (Table 3).

Stimulation with GtfD and S. mutans significantly increased IL-6 secretion by EOLP-T cells from 143 ± 94 to 12,614 ± 981 pg/ml ($p < 0.001$) and to 12,046 ± 828 pg/ml ($p < 0.001$), respectively (Table 4). THL significantly enhanced PHA- and TT-stimulated IL-6 production by EOLP-T cells from 1567 ± 927 to 10,017 ± 1182 pg/ml ($p < 0.001$) and 210 ± 152 to 10,532 ± 1224 pg/ml ($p < 0.001$), respectively. AHCC also significantly increased PHA- and TT-stimulated IL-6 production by EOLP-T cells from 1567 ± 927 to 10,142 ± 1030 pg/ml ($p<0.001$) and 210 ± 152 to 11,437 ± 1341 pg/ml ($p<0.001$), respectively (Table 4).

Table 1. Modulation of antigen-stimulated interleukin-2 (IL-2) production by Tien-Hsien liquid (THL) or active hexose-correlated compound (AHCC) in T cells isolated from 15 patients with erosive oral lichen planus

Antigen	IL-2 level (pg/ml, mean ± SEM)			
	No antigen	Antigen only	Antigen+THL (1:1000 dilution)	Antigen+AHCC (5 μg/ml)
PHA (1 μg/ml)	8 ± 2	59 ± 10	44 ± 11	25 ± 5[a]
TT (10 μg/ml)	8 ± 2	25 ± 9	9 ± 3	17 ± 6
GtfD (10 μg/ml)	8 ± 2	7 ± 2	5 ± 1	7 ± 2
Streptococcus mutans (2×10^5 CFU)	8 ± 2	5 ± 2	5 ± 1	7 ± 2

Comparison by Wilcoxon signed rank test between the antigen-only and antigen+AHCC groups with [a]$p < 0.05$.
PHA, phytohemagglutinin; TT, glutaraldehyde-inactivated tetanus toxoid; GtfD, glucosyltransferase.

Table 2. Modulation of antigen-stimulated interferon (IFN)-γ production by Tien-Hsien liquid (THL) or active hexose-correlated compound (AHCC) in T cells isolated from 15 patients with erosive oral lichen planus

Antigen	IFN-γ level (pg/ml, mean ± SEM)			
	No antigen	Antigen only	Antigen+THL (1:1000 dilution)	Antigen+AHCC (5 μg/ml)
PHA (1 μg/ml)	3 ± 2	72 ± 13[a]	89 ± 52	44 ± 9
TT (10 μg/ml)	3 ± 2	12 ± 6	28 ± 15	20 ± 11
GtfD (10 μg/ml)	3 ± 2	130 ± 17[a]	123 ± 41	278 ± 39[c]
Streptococcus mutans (2×10^5 CFU)	3 ± 2	86 ± 27[b]	31 ± 18	142 ± 33

Comparison by Wilcoxon signed rank test between the no-antigen and antigen-only groups with [a]$p < 0.001$ and [b]$p < 0.01$ as well as between the antigen-only and antigen+AHCC groups with [c]$p < 0.005$.

PHA, phytohemagglutinin; TT, glutaraldehyde-inactivated tetanus toxoid; GtfD, glucosyltransferase.

The spontaneously released IL-10 level by EOLP-T cells without antigen stimulation was too low to be detected (< 1 pg/ml). Significantly higher levels of IL-10 were produced by EOLP-T cells after stimulation with GtfD (44 ± 9 pg/ml, $p < 0.001$) and S. mutans (47 ± 11 pg/ml, $p < 0.001$) compared to the spontaneously released IL-10 produced by EOLP-T cells without antigen stimulation (< 1 pg/ml) (Table 5). THL significantly depressed GtfD- and S. mutans-stimulated IL-10 production by EOLP-T cells from 44 ± 9 to 16 ± 5 pg/ml ($p < 0.05$) and 47 ± 11 to 20 ± 7 pg/ml ($p < 0.05$), respectively (Table 5).

DISCUSSION

This study found that GtfD and S. mutans were more-potent antigens than PHA and TT for stimulating cytokine production by EOLP-T cells, except for stimulation of IL-2 secretion. THL not only significantly increased PHA- and TT-stimulated TNF-α and IL-6 production by EOLP-T cells, but also significantly decreased GtfD-stimulated IL-10 production and S. mutans-stimulated TNF-α and IL-10 production by EOLP-T cells. These findings suggest that THL is an immunomodulator that can either potentiate or suppress cytokine secretion by EOLP-T cells.

This study also demonstrated that AHCC not only significantly enhanced PHA- and TT-stimulated TNF-α and IL-6 production and GtfD-stimulated IFN-γ production by EOLP-T cells but also significantly reduced PHA-stimulated IL-2 production by EOLP-T cells. These findings indicate that AHCC

Table 3. Modulation of antigen-stimulated tumor necrosis factor (TNF)-α production by Tien-Hsien liquid (THL) or active hexose-correlated compound (AHCC) in T cells isolated from 15 patients with erosive oral lichen planus

Antigen	TNF-α level (pg/ml, mean ± SEM)			
	No antigen	Antigen only	Antigen+THL (1:1000 dilution)	Antigen+AHCC (5 μg/ml)
PHA (1 μg/ml)	6 ± 3	52 ± 9[a]	275 ± 32[b]	148 ± 42[c]
TT (10 μg/ml)	6 ± 3	9 ± 3	253 ± 29[b]	108 ± 16[b]
GtfD (10 μg/ml)	6 ± 3	396 ± 34[a]	289 ± 45	433 ± 32
Streptococcus mutans (2×10^5 CFU)	6 ± 3	491 ± 27[a]	311 ± 27[b]	451 ± 30

Comparison by Wilcoxon signed rank test between the no-antigen and antigen-only groups with [a]$p < 0.001$ as well as between the antigen-only and antigen+THL or antigen+AHCC groups with [b]$p < 0.001$ and [c]$p < 0.05$.

PHA, phytohemagglutinin; TT, glutaraldehyde-inactivated tetanus toxoid; GtfD, glucosyltransferase.

Table 4. Modulation of antigen-stimulated interleukin (IL)-6 production by Tien-Hsien liquid (THL) or active hexose-correlated compound (AHCC) in T cells isolated from 15 patients with erosive oral lichen planus

Antigen	IL-6 level (pg/ml, mean ± SEM)			
	No antigen	Antigen only	Antigen+THL (1:1000 dilution)	Antigen+AHCC (5 μg/ml)
PHA (1 μg/ml)	143 ± 94	1567 ± 927	10017 ± 1182[b]	10142 ± 1030[b]
TT (10 μg/ml)	143 ± 94	210 ± 152	10532 ± 1224[b]	11437 ± 1341[b]
GtfD (10 μg/ml)	143 ± 94	12614 ± 981[a]	10632 ± 893	12075 ± 951
Streptococcus mutans (2×10^5 CFU)	143 ± 94	12046 ± 828[a]	10663 ± 847	11929 ± 1031

Comparison by Wilcoxon signed rank test between the no-antigen and antigen-only groups with [a]$p < 0.001$ as well as between the antigen-only and antigen+THL or antigen+AHCC groups with [b]$p < 0.001$.

PHA, phytohemagglutinin; TT, glutaraldehyde-inactivated tetanus toxoid; GtfD, glucosyltransferase.

may also have the capability to modulate cytokine secretion by EOLP-T cells.

In this study, GtfD and S. mutans significantly increased IFN-γ, TNF-α, IL-6, and IL-10 secretion by EOLP-T cells. Our previous study showed that GtfD slightly augmented the proliferative response of EOLP-T cells compared to that of T cells from healthy control subjects[31]. Therefore, the significant GtfD-induced elevation of IFN-γ, TNF-α, IL-6 and IL-10 secretions may partially be attributed to an increase in the number of T cells that are capable of secreting these 4 cytokines after stimulation with GtfD. In addition, IFN-γ and TNF-α themselves can promote TNF-α synthesis and/or release from activated macrophages[15]. IFN-γ can induce the production of IL-6 mRNA.[36] TNF-α can induce the secretion of IFN-γ by T lymphocytes and can stimulate the secretion of IL-6 by activated macrophages[37]. IL-6 can also induce the production of TNF-α by activated monocytes[15]. Thus, the significant GtfD- and S. mutans-induced elevations of IFN-γ, TNF-α, and IL-6 secretion by EOLP-T cells can also be attributed to the reciprocal stimulation by these 3 cytokines on one another.

Streptococcus mutans is a common pathogen found in dental plaque on the surfaces of teeth[38]. In patients with EOLP, S. mutans may secondarily infect oral ulcerative lesions of EOLP through mucosal breaks. Therefore, S. mutans antigens and its secreted proteins such as GtfD may penetrate into the ulcerative oral mucosa and elicit specific immune reactions that exacerbate EOLP lesions. This study

Table 5. Modulation of antigen-stimulated interleukin (IL)-10 production by Tien-Hsien liquid (THL) or active hexose-correlated compound (AHCC) in T-cells isolated from 15 patients with erosive oral lichen planus

Antigen	IL-10 level (pg/ml, mean ± SEM)			
	No antigen	Antigen only	Antigen+THL (1:1000 dilution)	Antigen+AHCC (5 μg/ml)
PHA (1 μg/ml)	< 1	5 ± 1	5 ± 1	5 ± 1
TT (10 μg/ml)	< 1	< 1	3 ± 1	4 ± 2
GtfD (10 μg/ml)	< 1	44 ± 9[a]	16 ± 5[b]	37 ± 7
Streptococcus mutans (2×10^5 CFU)	< 1	47 ± 11[a]	20 ± 7[b]	48 ± 12

Comparison by Wilcoxon signed rank test between the no-antigen and antigen-only groups with [a]$p < 0.001$ as well as between the antigen-only and antigen+THL groups with [b]$p < 0.05$.

PHA, phytohemagglutinin; TT, glutaraldehyde-inactivated tetanus toxoid; GtfD, glucosyltransferase.

showed that THL significantly decreased GtfD-stimulated IL-10 production by EOLP-T cells and S. mutans-stimulated TNF-α and IL-10 production by EOLP-T cells. THL also slightly reduced GtfD-stimulated TNF-α, and IL-6 production by EOLP-T cells and S. mutans-stimulated IFN-γ and IL-6 production by EOLP-T cells, although the differences were not significant. Previous studies showed increased production of IL-2, IFN-γ, TNF-α, IL-6, and IL-10 by tissue-infiltrating mononuclear cells from OLP lesions[22,23], elevated unstimulated secretion of TNF-α and IL-6 by OLP-PBMCs[24], and augmented serum levels of TNF-α and IL-6 in OLP patients[8-10,24,25]. The results of our study indicate that THL can significantly or slightly decrease GtfD- and S. mutans-stimulated IFN-γ, TNF-α, IL-6, and IL-10 production by EOLP-T cells. Therefore, we suggest that THL may be a potential immunomodulator for treatment of OLP.

The reasons why THL has modulating effects on cytokine production by EOLP-T cells are still not very clear. IL-2 is a T-cell growth factor. Its major function is to enhance proliferation of activated T cells[15]. TNF-α has multiple stimulatory activities on activated T cells, including increasing the proliferation in response to antigens, increasing IL-2 receptor expression, and increasing the response to an IL-2 stimulus. IL-6 acts together with IL-2 to induce T-cell proliferation and cytotoxic T lymphocyte generation[15]. Furthermore, as stated before, IFN-γ, TNF-α, and IL-6 are closely related inflammatory cytokines, and one can induce production of the others[15]. Because previous studies on mice showed that ingredients of THL can induce secretion of IFN-γ and IL-2 by mouse spleen cells, increase the expression of IL-2R by murine lymphocytes, and induce the proliferation of murine lymphocytes[26,28,29], it was not difficult to explain how THL can significantly or slightly augment PHA- or TT-stimulated IFN-γ, TNF-α, and IL-6 production by EOLP-T cells. On the contrary, ingredients of THL can also decrease PHA-induced and antigen-stimulated IL-2 secretion by murine spleen cells and inhibit blast transformation and proliferation of murine lymphocytes[26,28,29]. Therefore, it is not difficult to understand how THL can significantly or slightly decrease GtfD- and S. mutans-stimulated IFN-γ, TNF-α, and IL-6 production by EOLP-T cells.

EOLP is probably a T-cell-mediated disease with elevated levels of IL-2, IFN-γ, TNF-α, IL-6, and IL-10 in either the patient's serum or oral lesions. Our study showed that GtfD- and S. mutans-stimulated IFN-γ, TNF-α, IL-6, and IL-10 production by EOLP-T cells could be significantly or slightly reduced by THL. Therefore, we suggest that THL may be a potential immunomodulator for treatment of EOLP.

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