Analysis of Immunologic Function Changes in Lichen Planus After Clinical Treatment

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Statistical Analysis C
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Background: Lichen planus (LP) is a common chronic superficial skin lesion that causes chronic or sub-acute inflammatory disorders. LP can affect the oral cavity, skin, mucous membrane, and other body parts, and features include repeat attacks and long duration, leading to lower quality of life. This study aimed to analyze the changes of immunologic function before and after treatment of LP.

Material/Methods: Thirty cutaneous LP patients were selected. Peripheral blood was collected in the morning before and after treatment. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient method. Flow cytometry was used to detect T cell subpopulation $CD4^+$ T cells and $CD8^+$ T to calculate $CD4^+$ $T/CD8^+$ T ratio. Enzyme-linked immunosorbent assay (ELISA) was adopted to detect the helper T-cell (Th) factor IL-2, IFN-γ, IL-4, IL-6, IL-17, and IL-22 levels.

Results: Compared with before treatment, the expressions of $CD4^+$ T cells and $CD8^+$ T cells were decreased, while the proportion of $CD4^+$ $T/CD8^+$ T were significantly elevated after treatment. IL-2 and IFN-γ secretion were markedly increased, whereas IL-4, IL-6, IL-17, and IL-22 were significantly reduced after treatment ($P<0.05$).

Conclusions: LP treatment reduces the distribution of $CD4^+$ T cells and $CD8^+$ T cells, and promotes the changes of Th1, Th2, and Th17 cytokines secretion.

MeSH Keywords: Cytokines • Lichen Planus • Paneth Cells

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Background

Lichen planus (LP), also known as flat red moss, affects the oral cavity, skin, mucous membranes, hair follicles, and nails. It is a chronic or subacute superficial skin lesion that is common in dermatology [1,2]. LP occurs mostly in middle-aged people between 50 and 60 years old, and mainly in women [3]. The clinical symptoms of LP vary, with multiple subtypes. Most lesions are characterized by small, purple-red, and polygonal flat papules. Wickham patterns can be observed. Some patients might have erosion, ulcers, and bullae, accompanied by burning sensation [4,5]. LP can result in repeat attacks with long duration sustainable for months to years, resulting in lower quality of life [6]. LP can be an idiopathic or secondary disease with complicated causes, including tumor, infection, and drug application. The whole process is multifactorial and has multigene participation. The etiology has not been fully elucidated [7]. The pathological changes of LP show hyperkeratosis of the epidermis, irregular thickening of the spine layer, and wedge-shaped granules in the epidermis with focal thickening, liquefaction degeneration of the basal cells, and a large number of lymphocyte infiltration in the dermis, which is an important basis for the diagnosis of LP [8,9].

At present, it is generally believed that the pathogenesis of LP is mediated by T cells and is associated with an abnormal immune response [10]. Changes in immune function are important reasons for the occurrence, development, and even recurrence of LP [11]. T cells are important immune cells in the body, and the distribution of T cells influences cellular immunity in immune response. Cellular immunity is particularly important in the progression of disease [12]. T cells can be divided into $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes based on their own markers. Changes in the distribution and proportion of T cells present a crucial impact on the occurrence and development of LP [13,14]. T cell subsets also include Th cells, which can be further divided into Th1 and Th2 subpopulations. Th1 and Th2 cytokine secretion imbalance is a key pathogenic factor. Thus, imbalance of cytokines secreted by Th1 and Th2 cells may the key factors leading to LP occurrence and progress [15,16]. Apart from lesional T cells, peripheral T cells might also play an important role in the pathogenesis of LP, as demonstrated by a decreased proportion of naïve T cells and an increased proportion of primed memory T cells [17], impaired spontaneous and mitogen-induced lymphocyte blastogenesis, as well as reduced secretion of T cell-related cytokines, such as IL-2, IL-6, IFN-γ, and lymphotoxin [18] in the peripheral blood of patients with LP. Therefore, this study intends to investigate the changes of immunologic function before and after treatment of patients with LP.

Material and Methods

General information

A total of 30 patients diagnosed with cutaneous LP in the Affiliated Hospital of Hebei University of Engineering from January 2017 to August 2017 were selected. All patients were diagnosed by clinical symptoms and pathology (biopsy) and confirmed by consensus of at least 2 dermatologists. There were 17 males and 13 females with mean age at 43±4.1 years (range 22–63 years) years. The papules were mainly distributed on the limbs, trunk, and neck. Some patients had accompanied pruritus and apathy. The course of disease ranged from 1 to 6 years. Patients with systemic disease, severe cardiovascular and cerebrovascular diseases, blood and endocrine system disorders, malignant tumors, and abnormal liver function were excluded [4]. This study was approved by the Medical Ethics Committee of the Affiliated Hospital of Hebei University of Engineering (Approval number: AHHUEEC20161016). All selected Participants had a signed informed consent. This was a pilot study and convenient sample size was taken.

Treatment method

All patients received 50 mg levamisole 3 times a day, once every 3 days with the study protocol as per the same oral levamisole was administered to all patients with reasons to justify the same decision. One course was sustained for 1 month, and the patients received a total of 2 to 3 courses.

Main reagents and instruments

Mouse anti-human CD3-APC monoclonal antibody, mouse anti-human CD4-FITC monoclonal antibody, mouse anti-human CD8-PE monoclonal antibody, fixing solution, and membrane disrupter were purchased from Miltenyi Biotec (Germany). RPMI1640 cell culture medium, fetal bovine serum (FBS), and Hank's solution were purchased from Invitrogen (USA). Human IL-2, IFN-γ, IL-4, IL-6, IL-17, and IL-22 ELISA kits were purchased from BD Biotech. Eppendorf 5524 centrifuge was purchased from Eppendorf (USA).The human lymphocyte stratified liquid was purchased from Tianjin Haoyang Biological Co., Ltd. Bechtop was purchased from Suzhou Antai Instrument Co., Ltd. The flow cytometer-EPICS XL was purchased from Beckman-Coulter (USA).

Methods

Sample collection

A total of 10 mL fasting blood was extracted from the elbow superficial vein at 24 hours within admission and after surgery. Then 5 mL were used to isolate human peripheral blood
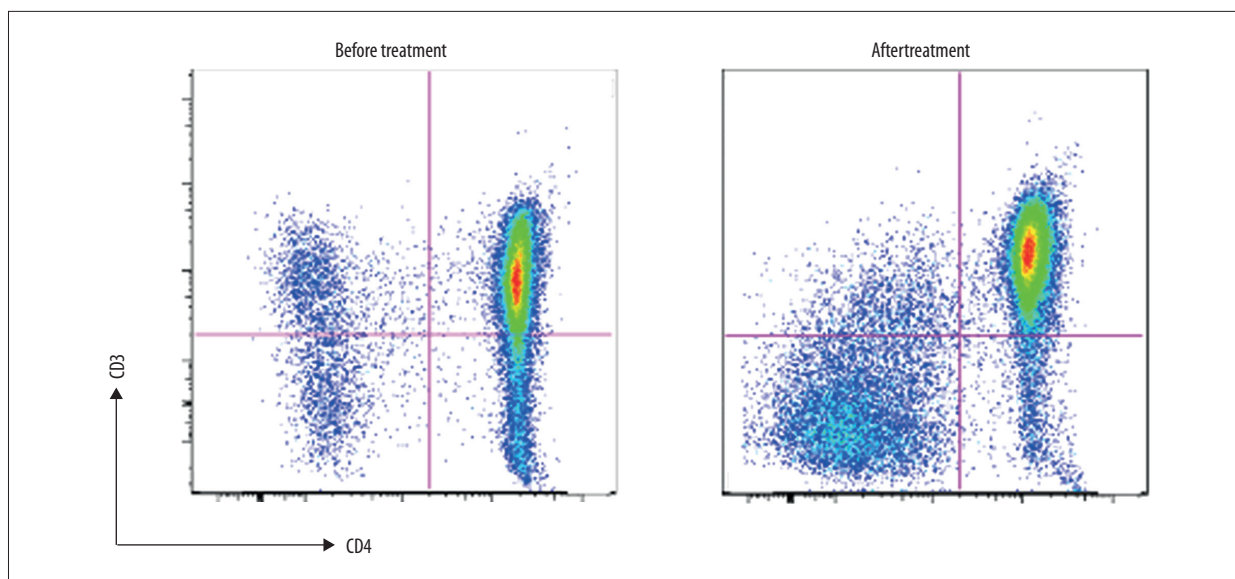
Figure 1. CD4+ T cell detection in lichen planus patients after treatment.

mononuclear cells (PBMCs). The remaining 5 mL was centrifuged at 3000 rpm for 15 min. The serum was placed in Eppendorf (Ep) tube and stored at −20°C for ELISA detection.

Flow cytometry

The PBMCs were centrifuged at 1000 rpm for 5 min and resuspended in 1.5 mL flow-washing solution. Then the cells were blocked by serum at 4°C for 15 min. After centrifugation at 1000 rpm for 5 min, the cells were incubated in 10 μL of CD3-APC, CD4-FITC, and CD8-PE at 4°C avoiding light for 30 min. After centrifugation, the cells were treated with 750 μL fixing solution, avoiding light for 20 min, and 1.2 mL membrane-destroying agent. At last, the cells were mixed in 300 μL flow-washing solution and detected on flow cytometry.

ELISA

The serum levels of IL-2, IFN-γ, IL-4, IL-6, IL-17, and IL-22 in each group were detected by ELISA. The collected peripheral blood was centrifuged, and the supernatant was taken. The experimental procedure was performed according to the ELISA kit instructions. The 50 μL diluted standard substance and samples were added to 96-well plate at 37°C for 30 min. After washing for 5 times, then 50 μL reagent A and 50 μL reagent B were added and plate incubated at 37°C for 10 min. At last, the plate had 50 μl stop solution added and was tested on a microplate reader. The standard curve was prepared based on the optical density (OD) value to calculate sample concentration.
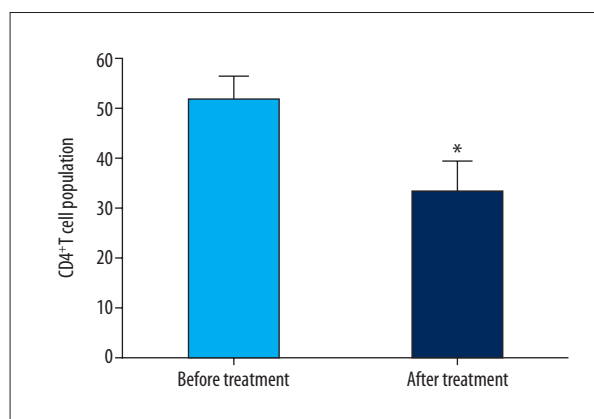

Figure 2. CD4+ T cell population changes in lichen planus patients after treatment. * $P<0.05$, compared with before treatment.

Statistical analysis

All data analyses were performed on SPSS 22.0 software. Measurement data were expressed as mean ± standard deviation (SD) and compared by one-way ANOVA or *t*-test. The test level was taken as α=0.05. *P*<0.05 was treated as statistical significance.

Results

CD4+ T cell distribution in LP patients after treatment

We isolated the blood PBMCs from LP patients before and after treatment to analyze CD4+ T cell distribution changes by flow cytometry. The results showed that compared with before
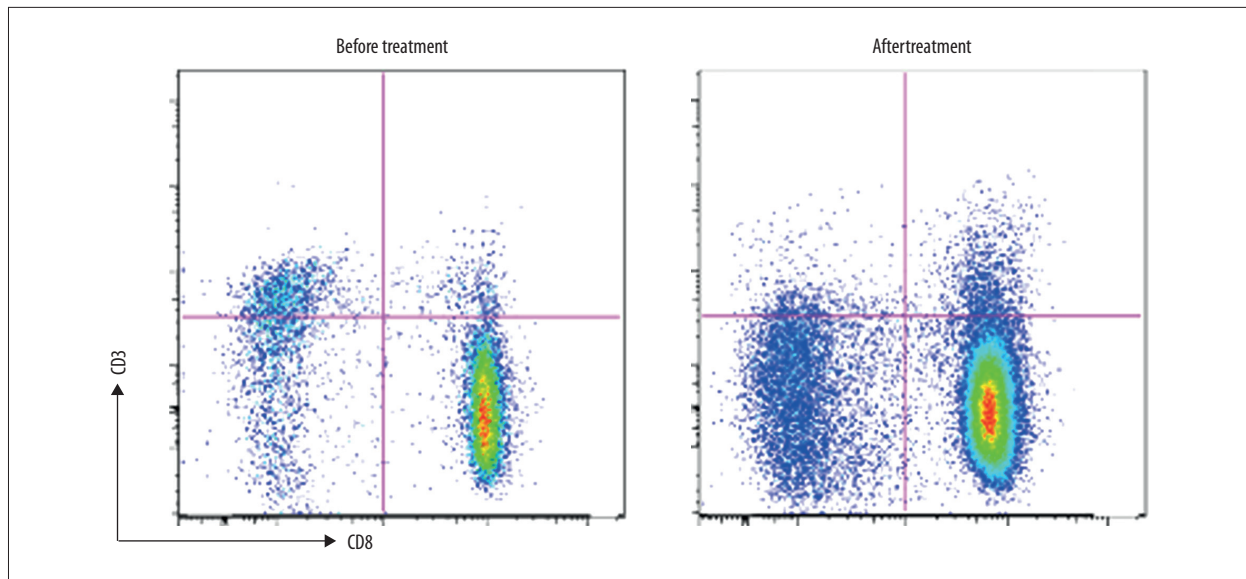
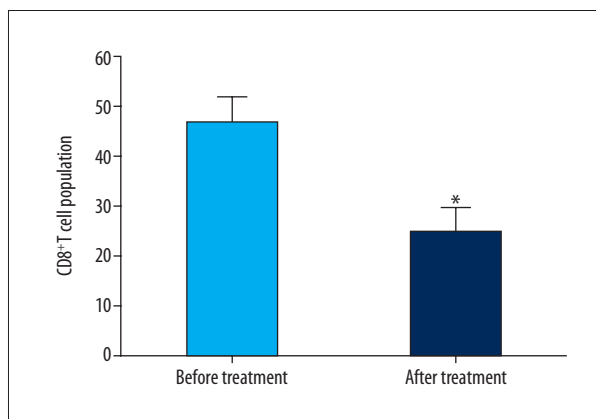**Figure 3.** CD8+ T cell detection in lichen planus patients after treatment.

Figure 4. CD8+ T cell population changes in lichen planus patients after treatment. * $P<0.05$, compared with before treatment.

treatment (51.9±0.6%), CD4+ T scope significantly decreased after treatment (36.3±0.4%) ($P<0.05$) (Figures 1, 2). This suggested that drug treatment can downregulate CD4+ T cells distribution in cutaneous LP patients.

CD8+ T cell distribution in LP patients after treatment

We isolated the blood PBMCs from LP patients before and after treatment to analyze CD8+ T cell distribution changes by flow cytometry. The results showed that compared with before treatment (48.1±0.7%), CD8+ T scope obviously decreased after treatment (25.3±0.3%) ($P<0.05$) (Figures 3, 4). This suggested that drug treatment can downregulate CD8+ T cells distribution in cutaneous LP patients.

CD4+ T/CD8+ T cell ratio changes in LP patients after treatment

We further analyzed the changes of CD4+ T/CD8+ T cell ratio in LP patients after treatment. The results revealed that compared with the before treatment (0.79±0.4), CD4+ T/CD8+ T cell ratio apparently elevated in the patients after treatment (1.35±0.6) ($P<0.05$) (Figure 5). The CD4+ T/CD8+ T cell ratio in patients after treatment was significantly improved.

Th1 cytokines IL-2 and IFN-γ expressions analysis in LP patients after treatment

Blood samples were collected to test IL-2 and IFN-γ expression changes after treatment. IL-2 (358.1±41.02 *vs.* 257.4±31.03 pg/mL) and IFN-γ (347.8±35.2 *vs.* 208.7±26.9 pg/mL) secretion increased after treatment compared with before ($P<0.05$) (Figure 6).

Th2 cytokines IL-4 and IL-6 expressions analysis in LP patients after treatment

Blood samples were collected to test IL-4 and IL-6 expression changes after treatment. IL-4 (211.3±39.6 *vs.* 318.7±51.03 pg/mL) and IL-6 (188.4±29.5 *vs.* 259.2±18.5 pg/mL) secretion reduced after treatment compared with before ($P<0.05$) (Figure 7).

Th17 cytokines IL-17 and IL-22 expressions analysis in LP patients after treatment

Blood samples were collected to test IL-17 and IL-22 expression changes after treatment. IL-17 (59.8±9.3 *vs.* 139.7±11.2 pg/mL)
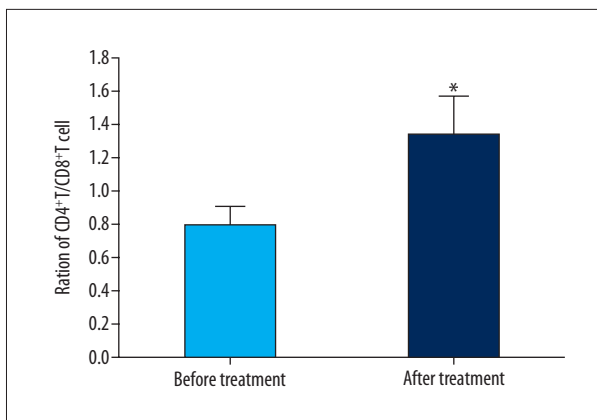
Figure 5. CD4+ T/CD8+ T cell ratio changes in lichen planus patients after treatment. * *P*<0.05, compared with before treatment.
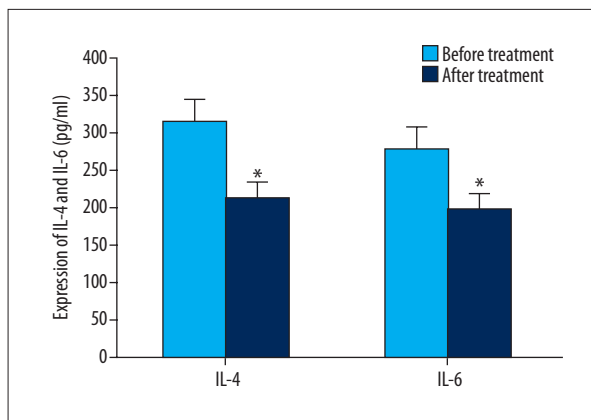

Figure 7. IL-4 and IL-6 expressions in lichen planus patients after treatment. * *P*<0.05, compared with before treatment.

Figure 6. IL-2 and IFN-γ expressions in lichen planus patients after treatment. * *P*<0.05, compared with before treatment.

Figure 8. IL-17 and IL-22 expressions in lichen planus patients after treatment. * *P*<0.05, compared with before treatment.

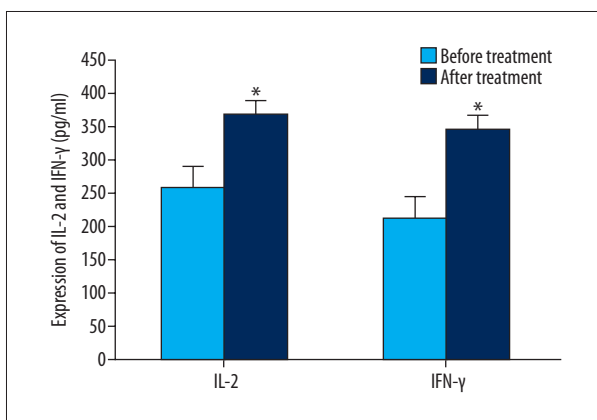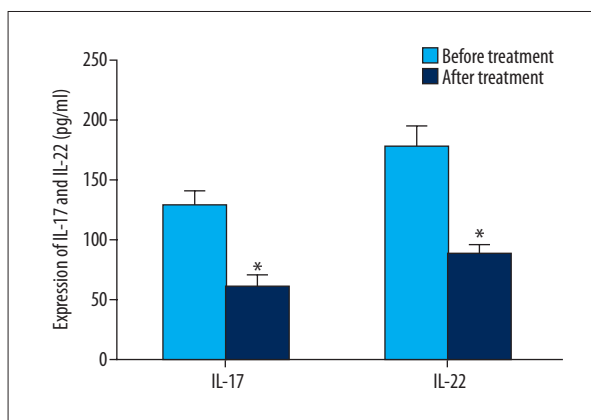and IL-22 (91.2±10.6 *vs.* 170.4±17.5 pg/mL) secretion declined after treatment compared with before (*P*<0.05) (Figure 8).

Discussion

Currently, it was showed that LP is a cell-mediated immune response and a type of autoimmune disease [19]. A large number of lymphocytic infiltrates, mainly T cells, can be detected at the site of lesion of the LP [20]. Numerous lymphocytes can be activated when local lesions occur at skin and mucous membranes, thereby increasing the secretion of various adhesion molecules and cellular kinases. B lymphocytes are rare in activated lymphocytes, and natural killer (NK) cells are uncommon. T lymphocytes are the main types [21,22]. CD4+ T cells and CD8+ T cells should be in dynamic equilibrium in normal immune states [23]. With the progression of LP, the increase in CD4+ T cells and CD8+ T cells is more pronounced. Therefore, LP is believed to be mainly caused by invasion of

the epidermis by CD4+ T cells and CD8+ T cells, killing epidermal cells and basal cells to accelerate the progression of the injury [24,25]. However, the changes in the expression of CD4+ T cells and CD8+ T cells after treatment of LP patients are not clear. This study showed that compared with before treatment, the expression of CD4+ T cells and CD8+ T cells decreased after treatment. The expression of CD8+ T cells reduced more significantly, and the proportion of CD4+ T/CD8+ T obviously increased, suggesting that the treatment of LP not only improves symptoms, but also effectively regulates the distribution of CD4+ T cells and CD8+ T cells.

Further research found that T cell subsets also include Th cells, which can be divided into Th1, Th2, and Th17 subpopulations [26], which can directly attack target cells expressing major histocompatibility antigens, and release a large number of cytotoxic factors to kill skin epidermal cells [27]. The Th2 cytokines IL-4 and IL-6 were significantly increased during the development of LP, and were strongly aggressive to normal

cells and caused damage [28]. In recent years, Th cells differentiated from CD4$^+$ T cells have been found to be differentiated into Th17 cells under the stimulation of some cytokines. Th17 cells secrete related cytokines IL-17 and IL-22 during the process of LP in the skin, which in turn mediated infiltration of dermatitis cells, damaged the basement membrane, and causing injury [29]. This study found that after treatment of LP, Th differentiation was altered. The secretion of Th1 cytokines IL-2 and IFN-γ were increased, Th2 cytokines IL-4 and IL-6 were decreased, and Th17 cytokines IL-17 and IL-22 secretion were reduced, which in turn inhibited tissue damage caused by cytokines. This study analyzed the immunological function of LP after treatment. However, due to limited number of the sample size, a large cohort clinical study is required to clarify the relevant mechanism of LP in clinic.

Conclusions

LP treatment reduces the distribution of CD4$^+$ T cells and CD8$^+$ T cells, and promotes the changes of Th1, Th2, and Th17 cytokines secretion.

Conflict of interest

None.

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