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ORIGINAL ARTICLE

The Expression of CXCL10/CXCR3 and Effect of the Axis on the Function of T Lymphocyte Involved in Oral Lichen Planus

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Abstract— The etiology of oral lichen planus (OLP) is still not clear. The purpose of this study was to explore the role of CXC chemokine receptor 3(CXCR3) and its ligand CXC motif chemokine 10(CXCL10) in the pathogenesis of OLP. We examined the expression of CXCR3 and CXCL10 in OLP patients and healthy controls by quantitative real-time PCR, Western blotting, ELISAs, and immunohistochemistry, respectively. Moreover, we detected the effects of CXCL10/CXCR3 axis on T lymphocyte migration, proliferation and apoptosis by Transwell assays, CCK8 assays, and flow cytometry. We found that the expression of CXCR3 and CXCL10 was significantly increased in OLP patients. In addition, T lymphocyte migration rate of CXCL10 stimulation group was significantly higher than that of control and CXCR3 antagonist groups. After antagonizing CXCR3, the migration ability of T lymphocytes was significantly decreased, and regardless of whether CXCL10 was added in the upper chamber culture medium, the number of migrating cells was similar. The addition of CXCL10 stimulant could stimulate the proliferation of T lymphocytes, but there was no significant difference compared with control group. After antagonizing CXCR3, the proliferation rate of T lymphocytes was significantly reduced. However, there were no significant differences in the apoptosis rates of T lymphocytes between CXCL10 stimulation group, antagonist CXCR3 group, and control group. Due to the change of expression in CXCR3 and CXCL10, and its interaction in mediating the directional migration of peripheral blood T lymphocytes, affecting the proliferation of T lymphocytes, it suggests that CXCL10/CXCR3 axis may be related to the immune mechanism of OLP.

KEY WORDS: CXCR3; CXCL10; oral lichen planus; chemokine.

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory oral mucosa disease characterized by a dense, band-like lymphocyte infiltration in the lamina propria associated with the degeneration of basal keratinocyte [1]. WHO has classified OLP as a premalignant condition [2] with a malignant transformation rate of 0.1–2% [3, 4]. The etiology and pathogenesis of OLP remain elusive. Previous studies have suggested that oral lichen planus should be

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considered a localized autoimmune disease mediated by T lymphocytes [5]. The imbalance in two subsets of T-helper cells, Th1 and Th2 cells, most likely contributes to the pathogenesis of OLP [6].

It is now well established that the differential expression of chemokines and chemokine receptors plays an essential role in mediating the trafficking of immune cells to sites of inflammation within both normal and inflamed tissue [7]. The chemokine receptor CXCR3 is a class A seven transmembrane-domain or G protein-coupled receptor (GPCR) that is involved primarily in the chemotaxis of certain immune cells, inhibition of angiogenesis, and polarization of Th1 cells [8–10]. Additionally, CXCR3 is predominantly expressed by the Th1 cell subset [11] and is associated with the pathophysiology of Th1-type diseases. CXCL10 that are produced in response to IFN-γ are allowed for the accumulation of activated lymphocytes by interacting with its specific receptor CXCR3 [12].

An abundance of data demonstrates that the CXCL10/CXCR3 axis plays important roles in many diverse autoimmune diseases, including autoimmune encephalomyelitis [13], thyroid autoimmune diseases [14], Graves' disease [15], and type 1 diabetes [16]. After binding to CXCR3, CXCL10 mainly participates in the generation and migration of effector T cells, and induces the directed migration of lymphocytes to specific sites of infection, causing the infiltration of lymphocytes, and ultimately producing local immune responses [17].

However, studies on whether the expression levels of CXCR3 and CXCL10 have changed and whether the CXCL10/CXCR3 axis has an effect on the function of T lymphocytes in patients with OLP are rare. The aims of this article are to explore the pathogenesis of OLP by detecting the levels of CXCR3 andCXCL10 and the effect of the CXCL10/CXCR3 axis on lymphocyte function in the peripheral blood of OLP patients.

MATERIALS AND METHODS

Volunteers for the Experiments Assessing the Expression of CXCR3/CXCL10 in the Peripheral Blood and Oral Tissue

Forty patients with OLP and 20 healthy controls (males 8, females 12, 44.80 ± 2.422) were enrolled in the study. The OLP patients were divided into two groups, the erosive group and non-erosive group, with 20 patients in each group (males 8, females 12, 43.50 ± 2.731; males 10, females 10, 46.60 ± 2.580). The clinical and histopathological diagnostic criteria for OLP patients met the guidelines set forth by the World Health Organization in 1978 and van der Meij et al. in 2003 [18]. The non-erosive group was defined in accordance with the Wickham striae on the oral mucosa with no painful, ulcerated and erythematous areas. The erosive group was mainly characterized by painful, ulcerated and erythematous areas, which reflected a more destructive phase of the disease.

The pathological biopsy of patients with OLP was taken from a representative area of the oral mucosal lesion, and all biopsies were performed prior to initial or systemic treatment. All healthy volunteer samples (including the peripheral blood and biopsy tissue) were admitted from orthognathic surgery. All tissue samples were immediately frozen in liquid nitrogen and stored at − 80 °C until further use.

Volunteers for the Experiments Assessing the Effects of CXCR3/CXCL10 on Cell Function in the Peripheral Blood

Eighteen patients with OLP (males 8, females 10, 43.00 ± 3.134) were enrolled in this part experiments with the same diagnostic criteria described above.

None of the healthy controls had oral mucosal diseases or autoimmune disease or had used antibiotics or immunologic agents for the past 3 months. In addition, OLP patients also have no other oral mucosal diseases and autoimmune diseases, and have not used antibiotics or immunologic agents for the past 3 months [19, 20].

The Expression of CXCR3, CXCL10 mRNA in the Peripheral Blood and CXCR3 mRNA in the Oral Tissue

The Isolation of Peripheral Blood T Lymphocytes

T lymphocytes were isolated from the peripheral blood by using Lymphoprep™ separation and an EasySepTM human CD3 positive selection kit (Stemcell Technologies, VAN, Canada) for further experiments. For the PMBC isolation, 10 ml fresh blood was diluted with phosphate-buffered saline (PBS)(Sigma-Aldrich, Munich, Germany) at a 1:1 ratio. Each sample has two tubes containing 5 ml diluted blood and 5 ml lymphocyte separation fluid for extracting RNA and protein. Then the tubes were centrifuged for 20 min at 2000 rpm. After centrifugation, the buffy coat was collected. The plasma was removed and stored at − 80 °C for further ELISAs. Next, the buffy coat was washed twice with PBS at 1000 rpm for 10 min, and the supernatant was discarded. Finally, we used a human CD3 positive

selection kit to isolate the T lymphocytes. The cells were placed in an incubator at 37 °C with 5% CO_2.

RNA Extraction and Quantitative Real-time Polymerase Chain Reaction

Total RNA was extracted from the T lymphocytes and oral tissues by using a Takara reagent kit (Takara, Tokyo, Japan). The purity and concentration of the RNA were determined by a Unico UV-2000(Unico, Shanghai, China). The RNA was reverse-transcribed into cDNA by using a PrimeScript RT reagent kit (Takara, Tokyo, Japan). RT-qPCR was performed with a quantitative PCR system (ABI 7300, USA) with a SYBR Green reagent. All primers were designed and synthesized by the same manufacturer (Invitrogen, Carlsbad, CA, USA). The following primers were used: CXCR3, forward primer: TTGTACCG ATTGCCTACTCCTT and reverse primer: CCCAGAAT GGGAGAGTAAGAAC; CXCL10, forward primer: AACTGTACGCTGTACCTGCAT and reverse primer: GCATCGATTTTGCTCCCCTC; and GAPDH, forward primer: ACCACAGTCCATGCCATCAC and reverse primer: TCCACCACCCTGTTGCTGTA. The expression level of the mRNA of the target gene was represented and calculated with the $2^{-\Delta\Delta Ct}$ method. All RT-qPCR experiments were done in duplicates and repeated three times to validate the results.

Western Blot Analysis

The protein expression of CXCR3 was detected by Western blotting. T lymphocytes in each group were homogenized by mixing with radioimmunoprecipitation assay buffer (RIPA buffer) (Beyotime, Shanghai, China) containing protease inhibitors (100:1). Proteins were extracted by 13680*g* centrifugation for 10 min and were quantified by the BCA method. All proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 10% polyacrylamide gels and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Next, the PVDF membranes were placed in 5% bovine serum albumin (BSA) to block for 2 h at room temperature. The blots were then incubated in primary antibody(1:1000, ab154845; Abcam, USA) overnight at 4 °C, washed with TBST, and incubated with secondary antibodies (1:1000; Zhongshan Golden Bridge, Beijing, China) for 1 h at room temperature. The blots were then washed again. Finally, the protein bands were detected by using Immobilon Western Chemiluminescent HRP substrate (Millipore, Billerica, MA, USA) and visualized by using the Image Quant LAS 4000 mini imaging system (GE, New York, USA). The results were analyzed by gray value *via* Compass software.

Enzyme-Linked Immunosorbent Assay

The concentration of CXCL10 was determined by using a human CXCL10 enzyme-linked immunosorbent assay (ELISA) kit (Multi Sciences, Hangzhou, Zhejiang, China) according to the manufacturer's instructions. The samples were diluted with a 1:1 ratio. Twenty plasma samples in each group were continuously tested for twice times, and three sets of duplicate wells were tested in parallel for each group in each time to obtain intra- and inter-assay coefficients of variation of CXCL10.

Briefly, monoclonal antibodies specific for CXCL10 has been pre-coated onto 96-well microtiter plates. Standards, samples and biotin-linked detection antibodies specific for CXCL10 were pipetted into the wells. CXCL10 was bound by the immobilized antibody and the detection antibody following incubation. After washing away any unbound substances, streptavidin-HRP was added. After washing, the substrate solution was added to the wells. Color develops in proportion to the amount of CXCL10 bound in the initial step. The color development was stopped and the intensity of the color was measured.

The Expression of CXCR3 and CXCL10 in the Oral Tissue

Immunohistochemistry

Tissue specimens were fixed in 10% neutral-buffered formalin for 24 h and then underwent standard tissue processing and embedding. Then, the specimens were cut into 4-μm thick sections and incubated onto microscope slides at 37 °C overnight. The sections were then incubated with a primary antibody (CXCL10: AF-266-NA, R&D Systems, Minneapolis, MN, USA; CXCR3: ab64714, Abcam, Cambridge, UK) overnight, which was followed by a secondary antibody incubation for 30 min. All the sections were counterstained by using hematoxylin and were dehydrated, cleared, and mounted before being examined with a microscope (DM4000B, Leica, Germany).

The positive signal appeared as brownish-yellow staining in the cell nuclears/cytoplasm. All slides were evaluated by two senior oral pathologists independently. The staining results were semi-quantitatively evaluated, based on the ratio of the staining intensity and proportion of positive cells. The intensity score was defined as 0, negative; 1, weak; 2, moderate; or 3, strong, brown. The

proportion score was defined as 0, negative; 1, < 10%; 2, 11–50%; 3, 51–80%; or 4, > 80% positive cells. The immunoreactive score was calculated as the intensity score × proportion score, and the specimens were divided into three groups based on the final score: 0, negative; 1–4, weakly positive expression; > 4, strongly positive expression [21].

The Effects of CXCL10/CXCR3 on the Migration of T Cells *In vitro*

Transwell Assay

Migration assays were performed by using a transwell chamber (5 μm diameter) with 5 × 10^5 cells/well. T cells extracted from the peripheral blood were divided into four groups according to different treatments: (1) control group (T cells were not treated); (2) CXCL10 group (T cells were added stimulant CXCL10); (3) anti-CXCR3 group (T cells were added anti-CXCR3 antagonist); and (4) anti-CXCR3 + CXCL10 group (T cells were incubated with anti-CXCR3 antagonists for 2 h followed by the addition of stimulator CXCL10). Briefly speaking, 600 μL of RPMI 1640 medium was added to a 24-well plate, and 0.9 μL/100 μL of CXCL10 was added to the CXCL10 group and the anti-CXCR3 + CXCL10 group, respectively. Two hundred microliters T cell suspension was added to the transwell chemotaxis chamber, and the anti-CXCR3 group and anti-CXCR3 + CXCL10 group received 1 μL/100 μL anti-CXCR3mAb. The transwell chemotaxis system was placed in 5% CO_2 and incubated for 2 h in a 37 °C incubator. The chemotaxis chamber was placed in a 24 well plate of RPMI 1640 medium and incubated for 6 h in an incubator. Then, aspirate the small indoor solution washed by PBS twice, fix it with 4% paraformaldehyde for 30 min, and stained with 60 μL crystal violet for 5 min. Finally, the number of cells was counted with a microscope. Three sets of duplicate wells were tested in parallel for each group, and the numbers of cells migrating to the back of the polycarbonate membrane in 5 randomly selected high-powered fields were counted. In addition, the cells penetrating into the lower chamber were counted, and the total number of cells was added to calculate the cell migration rate using the formula: (Mobility) = (total number of migrated cells in the experimental group - total number of migrated cells in the control group) / total number of migrated cells in the control group.

The Effects of CXCL10/CXCR3 on the Proliferation and Apoptosis of T Cells *In vitro*

T cells extracted from the peripheral blood were divided into three groups according to the different treatments: (1) control group (T cells were not treated); (2) CXCL10 group (T cells were added stimulant with CXCL10); (3) anti-CXCR3 group (T cells were added with anti-CXCR3 antagonist).

CCK8 Assay

Cell proliferation was measured by a CCK8 cell proliferation assay (Dojindo, Kyushu, Japan). T cells were plated (4 × 10^4 cells/well, 100 μl/well) in 96-well plates and then cultured at 37 °C for 24 h. Then CXCR3 and CXCL10 were added to each group at concentrations of 1 μL/100 μL, 0.9 μL/100 μL, respectively. Ten microliters of CCK8 reagent was added to each group well at 2 h, 24 h, 48 h, and 72 h under light protection, and incubated in the cell culture incubator for 4 h in the dark. The absorbance of the cells in each well was observed at 450 nm with an absorption spectrophotometer (Multiskan MK3, Thermo, Waltham, MA, USA). Three complex wells were used for each group, and the experiment was repeated three times to obtain the mean. The growth curves were illustrated by using GraphPad Prism 6 software.

Annexin V-FITC/PI Double-Color Fluorescent Labeled Flow Cytometry

Annexin V-FITC/PI detection kit (BD, New Jersey, USA) was used to detect apoptosis by following the manufacturer's instructions. Briefly, the harvested cells were resuspended in binding buffer at a density of 5×10^5 cells/mL. Each sample was mixed with 3 μL Anti-Annexin V-FITC antibody and 2 μL PI. The samples were kept in the dark at room temperature for 15 min. Flow cytometry (FACS-400, BD, New Jersey, USA) was used to detect the fluorescence. The rates of apoptosis were expressed as the number of Annexin V (+)/PI (−) cells and the number of Annexin V (+)/PI (+) cells.

Statistical Analysis

All experiments were carried out three times independently. The results are expressed in terms of the mean ± standard deviation (SD) values, and were tested for statistical differences by one-way ANOVA. $P < 0.05$ was considered significant.

RESULTS

The mRNA Levels of CXCR3 in the Peripheral Blood and Oral Tissue and the mRNA Levels of CXCL10 in the Peripheral Blood

The expression of CXCR3 mRNA in the peripheral blood and oral tissue of OLP patients was higher than that of the normal control group (Fig. 1a, $P < 0.05$; Fig. 1b, $P < 0.05$), but there was no significant difference between the non-erosive group and the erosive group ($P > 0.05$).

The expression of CXCL10 mRNA in the peripheral blood of OLP patients was higher than that of the normal control group ($P < 0.001$), but there was no significant difference between the non-erosive group and the erosive group ($P > 0.05$) (Fig. 1c).

The Protein Levels of CXCR3 and CXCL10 in the Peripheral Blood

The expression of CXCR3 and CXCL10 protein in the peripheral blood of OLP patients was higher than that of the normal control group (Fig. 2a, $P < 0.01$; Fig. 2b, $P < 0.01$), but there was no significant difference between the non-erosive group and the erosive group ($P > 0.05$). The intra- and inter-assay coefficients of variation of CXCL10 were 2.36~5.66% and 2.96~8.95%, respectively (Table 1).

The Distribution of CXCR3 and CXCL10 in the Oral Tissue

We detected CXCR3 and CXCL10 expression in the oral mucosa tissue by immunohistochemical analysis. The results showed that CXCR3 was weakly expressed in the membrane and cytoplasm of lymphocytes in the lamina propria of OLP patients. In the normal oral mucosa, CXCR3 was hardly expressed (Fig. 3a). The immunoreactive scores showed that the expression level of CXCR3 in the tissue lesions of patients with OLP was significantly higher than that of the normal control group ($P < 0.001$) (Fig. 3c). CXCL10 was strongly expressed in the membrane and cytoplasm of the lamina propria lymphocytes and the cell membrane of the epithelial cells in OLP patients. In the normal oral mucosa, the CXCL10 staining was weakly positive (Fig. 3b). The immunoreactive scores showed that the expression level of CXCL10 in the tissue lesions of patients with OLP was significantly higher than that of the normal control group ($P < 0.001$) (Fig. 3d).

The Effects of CXCL10/CXCR3 on the Migration of T Cells *In vitro*

The migration rate of T cells in the CXCL10 group was significantly higher than that in the control group and anti-CXCR3 group ($P < 0.001$). After the CXCR3 gene was antagonized, the migration ability of the T cells was significantly decreased ($P < 0.01$), and regardless of whether CXCL10 was added to in the

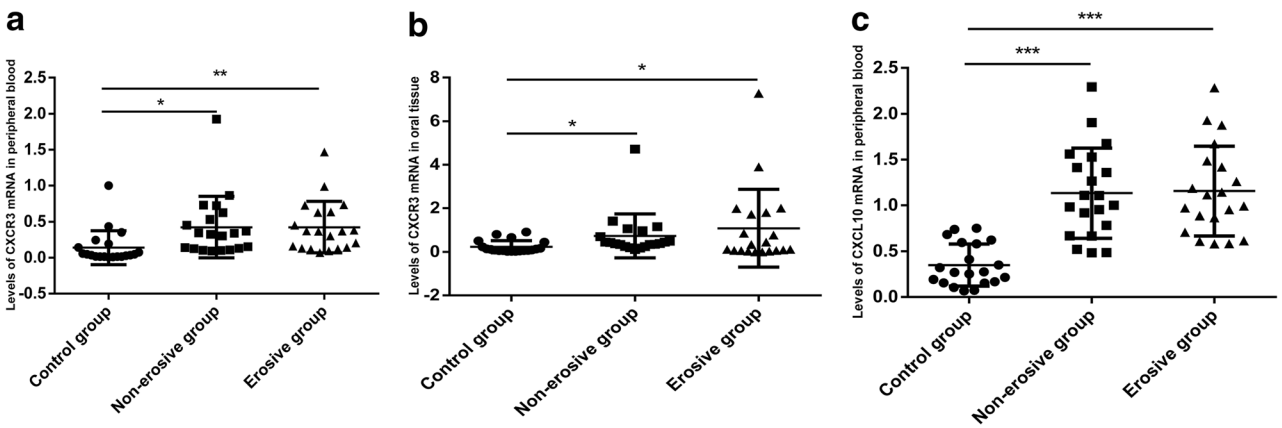

Fig. 1. **a** The expression of CXCR3 mRNA in the peripheral blood of the non-erosive group and erosive group was higher than that of the normal control group (*, $P < 0.05$; **, $P < 0.01$), but there was no significant difference between the non-erosive group and erosive group ($P > 0.05$). **b** The expression of CXCR3 mRNA in oral tissue of the non-erosive group and erosive group was higher than that of the normal control group (*, $P < 0.05$), but there was no significant difference between the non-erosive group and erosive group ($P > 0.05$). **c** The expression of CXCL10 mRNA in the peripheral blood of the non-erosive group and erosive group was higher than that of the normal control group (***, $P < 0.001$), but there was no significant difference between the non-erosive and erosive groups ($P > 0.05$).

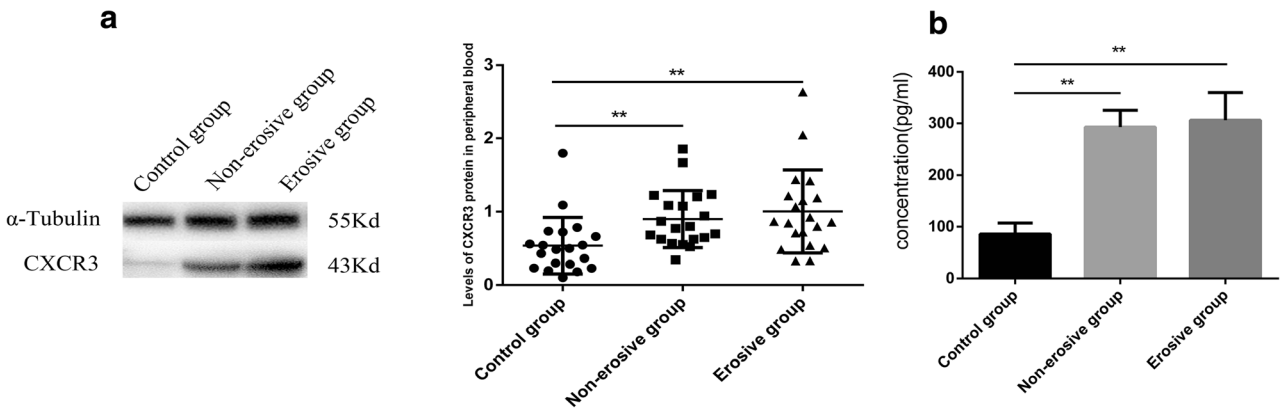

Fig. 2. **a** The expression of CXCR3 protein in the peripheral blood of the non-erosive group and erosive group was higher than that of the normal control group (**, $P < 0.01$), but there was no significant difference between the non-erosive group and erosive group ($P > 0.05$). **b** The expression of CXCL10 protein in the peripheral blood of the non-erosive group and erosive group was higher than that of the normal control group (**, $P < 0.01$), but there was no significant difference between the non-erosive group and erosive group ($P > 0.05$).

upper chamber culture medium, the number of migrating cells was similar ($P > 0.05$) (Fig. 4).

The Effects of CXCL10/CXCR3 on the Proliferation and Apoptosis of T Cells *In vitro*

T lymphocyte proliferation was stimulated in the groups treated with CXCL10 compared with the control group, but the difference was not significant ($P > 0.05$). The proliferation rate of T cells in the anti-CXCR3 group was significantly lower than that in the control group and CXCL10 group ($P < 0.05$) (Fig. 5).

There was no significant difference in the apoptosis rate of the T cells between the CXCL10 group, anti-CXCR3 group, and control group ($P > 0.05$) (Fig. 6).

DISCUSSION

A large number of studies have shown that immune factors play important roles in the occurrence and development of oral lichen planus. Immune dysfunction mediated by T cells is an important factor in the pathogenesis of OLP [22]. However, the mechanism behind the abnormally intensive infiltration of T lymphocytes in OLP is unclear. In recent years, the roles of chemokines and their receptors in immune diseases have gradually attracted attention [15]. The interaction of the chemokine receptor CXCR3 with its ligand CXCL10 has become a breakthrough point in the study of certain immune diseases.

There have been reports on the relationship between CXCL10/CXCR3 and various inflammatory skin diseases (such as lichen planus and chronic discoid lupus erythematosus). Chemokines and their receptors have been found to play important roles in T cell recruitment and infiltration in skin disease studies [23]. CXCR3 belongs to the CXC-type chemokine receptor class, and studies have shown that CXCR3 is selectively expressed on activated T cells, mainly Th1 cells, and not expressed on the rested T cells, B cells, monocytes, granulocytes and other related cells [24].

Table 1. The Intra- and Inter-Assay Coefficients of Variation for CXCL10

Sample	N	Mean		Standard deviation		Coefficient of variation /%	
Intra-assay variation		Range	Average	Range	Average	Range	Average
Control group	20	0.026~0.084	0.04995	0.001~0.009	0.0026	1.19~23.07	5.66
Non-erosive group	20	0.173~0.288	0.22775	0.001~0.008	0.0055	0.4~7.95	2.36
Erosive group	20	0.163~0.333	0.23995	0.001~0.034	0.01155	0.3~15.8	5.21
Inter-assay variation							
Control group	20	0.053225		0.004773		8.95	
Non-erosive group	20	0.232625		0.00689		2.96	
Erosive group	20	0.245975		0.00859		3.49	

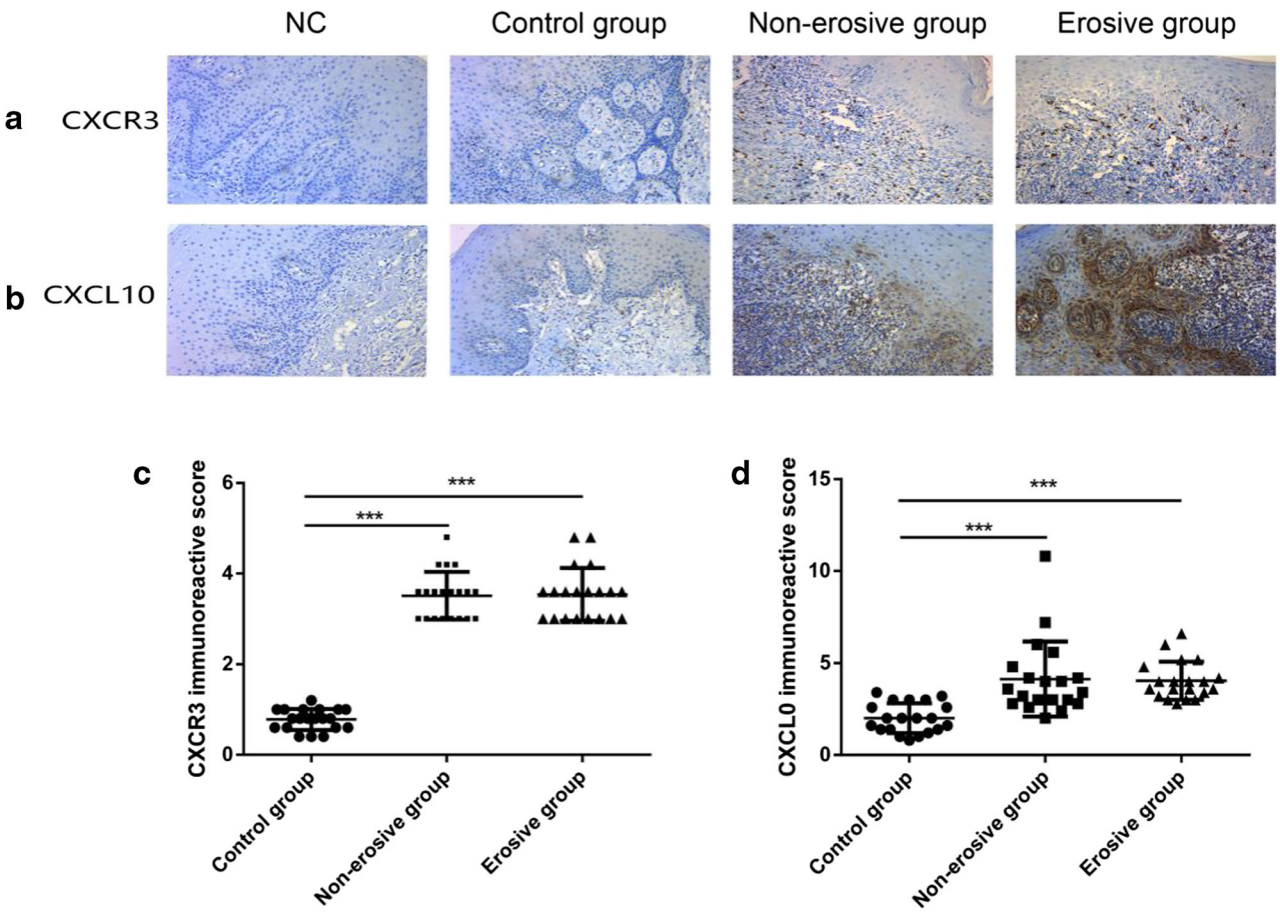

Fig. 3. **a** In the negative control group, CXCR3 was not expressed at all. In the normal oral mucosa, CXCR3 was hardly expressed. CXCR3 was weakly expressed in the membrane and cytoplasm of lymphocytes in the lamina propria of the non-erosive group and erosive group. **b** In the negative control group, CXCL10 was not expressed at all. In the normal oral mucosa, CXCL10 staining was weakly positive. CXCL10 was strongly expressed in the membrane and cytoplasm of the lamina propria lymphocytes and the cell membrane of the epithelial cells in the non-erosive group and erosive group. **c** The expression level of CXCR3 in the tissue lesions of the patients with OLP was significantly higher than that of the normal control group (***, $P < 0.001$). **d** The expression level of CXCL10 in the tissue lesions of the patients with OLP was significantly higher than that of the normal control group (***, $P < 0.001$).

Interferon-induced mononuclear factor (Mig), interferon-induced protein 10 (IP-10) (CXCL10), and interferon-induced T cell chemoattractant a (I-TAC) are all highly selective and high-affinity ligands for CXCR3. Among them, CXCL10 is mainly induced by interferon (IFN-y) and has many biological effects, such as recruiting of neutrophils, promoting cytokine secretion and partially inhibiting tumor growth [25]. It can bind to the receptor CXCR3 and recruit a large number of activated effector cells into the lesion site to eliminate inflammatory cells, but at the same time, due to the excessive release of chemokines, the inflammatory reaction is enlarged, and tissue damage is aggravated [26]. Therefore, the CXCL10/CXCR3 axis plays an important role in lymphocyte homing under physiological conditions and lymphocyte recruitment under pathological conditions.

In this study, we found that the expression of CXCR3 was significantly enhanced in the peripheral blood T lymphocytes of patients with OLP. This may be due to stimulation by antigens or other factors; a large number of initial T cells were activated in the peripheral blood of OLP patients, and cytokines, such as γ-interferon, were secreted to stimulate keratin production. The formation of activated cells, which in turn produce cytokines, promotes the activation of T cells through autocrine cytokines, thereby upregulating the expression of cell surface chemokine receptors. At the same time, due to the increased secretion of cytokines, such as γ-interferon, more CXCL10

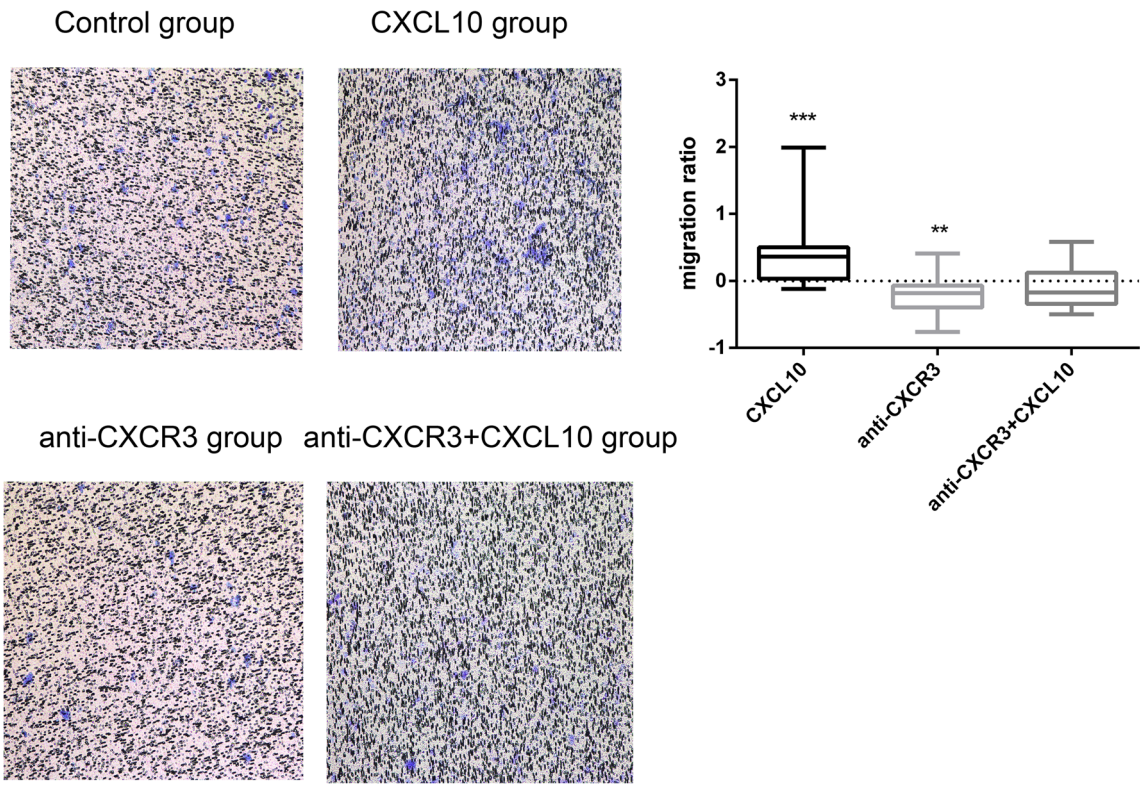

Fig. 4. The migration rate of T cells in the CXCL10 group was significantly higher than that in the control group and anti-CXCR3 group (***, $P < 0.001$). After the CXCR3 gene was antagonized, the migration ability of the T cells was significantly decreased (**, $P < 0.01$), and regardless of whether CXCL10 was added in the upper chamber culture medium, the number of migrating cells was similar ($P > 0.05$).

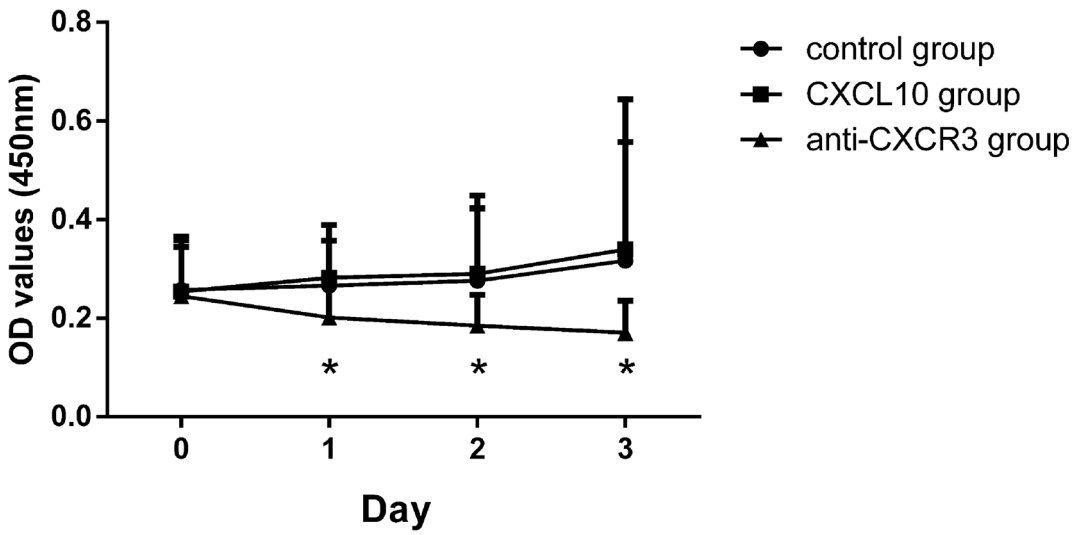

Fig. 5. Compared with the control group, the addition of CXCL10 could stimulate T lymphocytes proliferation, but there was no significant difference between the two groups ($P > 0.05$). The proliferation rate of T cells in the anti-CXCR3 group was significantly lower than that in the control group and CXCL10 group (*, $P < 0.05$).

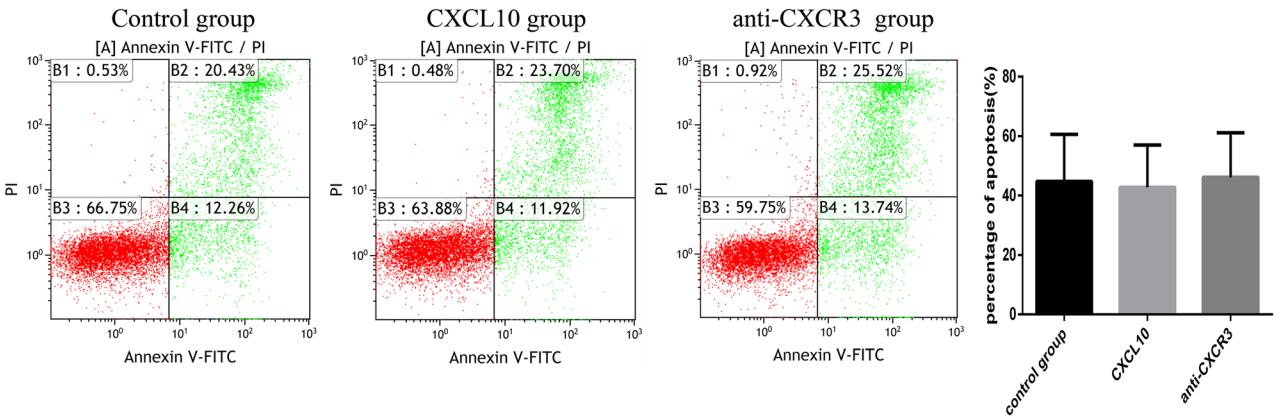

Fig. 6. There were no significant differences in the apoptosis rate of T cells between the CXCL10, anti-CXCR3 and control groups ($P > 0.05$).

expression can be induced. The results of this study confirmed that the mRNA and protein expression levels of CXCL10 in the peripheral blood of OLP patients were significantly increased. The pathological features of OLP include dense infiltration of subepithelial lymphocytes and liquefaction denaturation of basal cells. Due to the high expression of chemokine receptors, the corresponding expression levels of the ligands are also increased, which may "attract" activated T lymphocytes from the primary site into the blood circulation and transfer them to the local lesions [27], resulting in a large amount of T lymphocyte infiltration and basal cell damage. These aggregated lymphocytes secrete more cytokines (such as TNF-a and IFN-y) to induce the abnormal proliferation and activation of keratinocytes and secrete CXCL10, attracting more activated T cells into the lesions, resulting in a T cell-mediated malignant cycle that leads to the maintenance, progression, and prolongation of the disease. The results of the immunohistochemical analysis confirmed that CXCR3 was weakly expressed in the lymphocytes that infiltrated the lamina propria of OLP patients, but it was hardly expressed in the normal control group. In addition, CXCL10 was strongly expressed not only in the lamina propria of OLP patients, but also in the cell membrane of the epithelial keratinocytes. This is consistent with previous studies, and it is believed that the tissue damage seen in rheumatoid arthritis, multiple sclerosis, and other autoimmune diseases is associated with the accumulation of high CXCR3-expressing Th1 cells in the inflammatory site [28, 29].

To further investigate the role of CXCL10/CXCR3 in the OLP immune response, this study found that the migration index of T cells in the CXCL10-stimulated group was significantly higher than that in the control group *in vitro*. When the CXCR3 receptor was antagonized, the migration index of the T cells dropped significantly. However, after antagonizing CXCR3, there was no difference in the mobility of lymphocytes with or without CXCL10 treatment. This result confirmed that CXCR3 is a specific receptor for CXCL10 and that CXCL10 induces the chemotaxis of lymphocytes in oral lichen planus by binding to CXCR3. In a study on the pathogenesis of multiple sclerosis, researchers found that the increased secretion of CXCL10 can enhance the chemotaxis of T lymphocytes, promote the migration of T lymphocytes to the lesion site, and participate in the development of skin lesions [30]. In another molecular mechanism study exploring effects in immune-mediated hepatitis C, researchers used a mouse model of hepatitis C to analyze the expression of chemokines and their receptors during liver injury and found that after treatment with an anti-CXCR3 antibody, the number of T lymphocytes that migrated to the liver was reduced, thereby reducing liver damage [31]. At the same time, in Rasmussen encephalitis [27], active celiac disease [32], type I diabetes [33], and other diseases, it was found that the CXCL10/CXCR3 axis can play a role in the pathogenesis of the disease by affecting cell migration and aggregation.

This study also found that the proliferation of T lymphocytes after stimulation with CXCL10 stimulator was enhanced compared with the control group, but did not reach statistical difference. The proliferation of T lymphocytes was significantly inhibited after CXCR3 was blocked by antagonists. These observations also support the results obtained in the migration experiment. It is also

suggested that since CXCR3 has three ligands, CXCL10 may not be the main ligand of CXCR3, and only increasing the CXCL10 levels could not significantly enhance cell proliferation. Previous studies have shown that the signal transmitted by CXCL10/CXCR3 was blocked when CXCR3, the only receptor for CXCL10 was antagonized, suggesting that the CXCL10/CXCR3 axis was associated with T lymphocyte activity and immunity during OLP development and progression. In this process, CXCR3 plays a main role, and the combination of CXCR3 and CXCL10 allows T lymphocytes to proliferate and become activated, thereby producing an immune effect, mediating the occurrence and development of the disease.

In previous reports, Ha et al. found that retinal ganglion cells showed increased apoptosis under CXCL10 stimulation, and they could effectively reduce retinal ganglion cell damage by using a CXCR3 antagonist. The CXCL10 / CXCR3 axis has been shown to play an essential role in the induction of retinal ganglion cell death [34]. He et al. used CXCR3 antagonists to observe islet cell apoptosis in a rat model of type I diabetes, and found that the apoptosis of islet cells decreased significantly after antagonist treatment [35]. Abubaker et al. studied the effect of CXCL10 on apoptosis or survival in activated human primary T lymphocytes *in vitro*, and found that CXCL10 combined with IL-2 and/or IFNα induced T lymphocyte apoptosis. In addition, CXCL10-induced CXCR3 activation also triggers a pro-survival signal. The analysis of downstream signaling pathways indicated that apoptosis is p38 MAPK-dependent, and pro-survival signaling is dependent on the sustained activation of PI3K and transient activation of Akt [36]. However, in this study, it was found that the stimulation of CXCL10 and antagonism of CXCR3 had no effect on T lymphocyte apoptosis. We speculate that on the one hand, the CXCL10/CXCR3 axis has different abilities to promote or inhibit apoptosis in different cell types; on the other hand, T lymphocytes in OLP patients are activated and in a disease state, which is different from the response to CXCL10/CXCR3 in peripheral blood primary T lymphocytes in healthy normal subjects.

Supporting experiments have shown that the CXCL10/CXCR3 axis mainly recruits and directs T lymphocytes to the lesion site in OLP, and CXCR3 occupies a key role in this process. The proliferative ability of T lymphocytes was greatly affected by CXCR3. However, this axis has no effect on the apoptosis of T lymphocytes. Previous studies on the regulation of leukocyte recruitment to inflammatory sites by chemokines and their receptors suggested that the use of biologically targeted therapies may have the effect of downregulating the inflammatory response. Recent studies have shown that the adjuvant treatment of arthritis in rats with CXCL10 DNA vaccine could result in rapid and significant disease improvement. In addition, anti-CXCR3 antibodies were also found to inhibit the recruitment of Th1 lymphocytes to peripheral inflammatory sites in a mouse model. Our results provide evidences for the functional role of CXCR3/CXCL10 in mediating T cell recruitment to the inflammatory site in OLP.

Studies have found that differences in the type and expression of chemokines are important factors in regulating Th1/Th2 activation and polarization. Studies have confirmed that the pathogenesis of OLP is related to the imbalance in the Th1/Th2 ratio. Chemotaxis induced by CXCL10 binding to CXCR3 is highly selective for the recruitment of Th1 cells to the site of inflammation. Activated Th1 cells produce a large number of type I cytokines, such as interferon-γ, interleukin-2, and tumor necrosis factor-alpha. On the one hand, due to the balance of Th1/Th2 in the body being broken, an immune disorder appeared. On the other hand, the CXCR3 + Thl cells, cytokines, and cytokine receptor-mediated inflammatory networks were amplified step by step, resulting in repeated OLP-promoting conditions, difficulty healing, and chronic disease prolongation.

In summary, changes in the expression of CXCL10 and CXCR3 and the axis play a crucial role in the progression of oral lichen planus, possibly by upregulating the expression of CXCL10 and CXCR3 or regulating the proliferation and migration of T lymphocytes. This in turn triggers lamina propria lymphocyte aggregation. This study provides a new idea and target for studying the pathogenesis and clinical treatment of OLP.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: FY. Performed the experiments: FJ and WC. Analyzed the data: SC, SJ, WX, and LL. Wrote the paper: FJ and WC.

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COMPLIANCE WITH ETHICAL STANDARDS

Informed Consent. All experiments were informed consent by participants and approved by the Ethics Committee of Nanjing Medical University, Nanjing, China. (2014-132)

Conflict of Interest. The authors declare that they have no competing interests.

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