Implications of Th1 and Th17 Cells in Pathogenesis of Oral Lichen Planus

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Summary: Oral lichen planus (OLP) is considered a T cell-mediated autoimmune disease with unknown aetiology. T helper cells appear to play an important role in the pathogenesis of OLP. We investigated the possible role of T helper cells, Th1 and Th17, in the lesions and circulation of patients with OLP. Forty patients with OLP and 15 healthy volunteers were recruited. Double immunofluorescence staining was used to detect Th1 and Th17 cells in the OLP lesions, and intracellular cytokine staining and flow cytometry to evaluate the proportion of Th1 and Th17 cells in peripheral blood. The levels of serum interferon (IFN)-γ and interleukin (IL)-17 were assessed by using an enzyme-linked immunosorbent assay. It was found that Th17 cells, as well as Th1 cells, were present in OLP lesions. The proportion of peripheral Th1 and Th17 cells was significantly increased in patients with OLP. The proportion of Th17 cells in atrophic-erosive OLP was elevated as compared with that in reticular OLP. Serum IL-17 levels in OLP patients were significantly higher than in controls, and those in the atrophic-erosive OLP group were increased as compared with the reticular OLP group. However, the levels of serum IFN-γ were slightly decreased in OLP patients. Our data suggested that Th1 and Th17 cells in the local lesions and peripheral blood may be associated with the pathogenesis of OLP, and that IL-17 may be an important proinflammatory cytokine in OLP. These findings enhance our understanding of OLP pathogenesis.

Key words: interferon-γ; interleukin-17; oral lichen planus; Th1; Th17

Lichen planus is a chronic inflammatory disease mainly affecting the skin and mucosa with unknown aetiology. Oral lichen planus (OLP) presents as oral mucosal lesions such as white striation, erythema, erosion, papules, plaques, ulcers or blisters11. Previous studies have indicated that OLP is a T cell-mediated autoimmune disease characterized by a dense subepithelial lymphohistiocytic infiltration, increased number of intra-epithelial lymphocytes and degeneration of basal keratinocytes2. Although the majority of intra-epithelial lymphocytes and subepithelial lymphocytes are cytotoxic CD8+ T lymphocytes, in OLP, most lymphocytes in the lamina propria are identified as CD4+ T cells2, 3. CD4+ helper T cells have been suggested to play an important role in cytotoxic CD8+ T cell activation via T helper cell-related cytokine release, with activated autocrine toxic CD8+ T cells then triggering keratinocyte apoptosis2. It is well accepted that two CD4+ helper T cells subsets, namely Th1 and Th2, are associated with the immunopathogenesis of OLP. Th1 and Th2 cells are identified by their signature cytokines, interferon (IFN)-γ and interleukin (IL)-4, respectively. There is a mixed Th1 and Th2 cytokine profile in local inflammatory microenvironments that displays a Th1 cytokine bias5, 4. Therefore, Th1 cells have been implicated in the activation of cytotoxic CD8+ T lymphocytes through IFN-γ production5. A more recent study demonstrated that T-bet, the master transcription factor of Th1, is increased significantly in the peripheral blood of patients with OLP6, 7. Hence, the proportion of Th1 cells in the peripheral blood of OLP patients requires investigation.

Recently, Th17 cells (IL-17-producing CD4+ T cells) have been discovered as a unique subset of T helper cells that develop along a pathway that is distinct from the Th1 and Th2 differentiation pathways. Th17 cells are characterized by the production of the potent proinflammatory cytokines IL-17, IL-17F, IL-21, IL-22 and IL-26, which suggests that these cells function as pleiotropic proinflammatory T helper cells6, 7. The presence of Th17 cells and their signature cytokine, IL-17, has been demonstrated in autoimmune pathology, infectious inflammatory states and tumour microenvironments8, 9. Published data provide evidence of a higher proportion of Th17, but not Th1, in the skin lesions of patients with psoriasis, a common lichenoid tissue reaction/interface dermatitis (LTR/IFD) disorder10–12. However, studies of Th17 in the pathogenesis of lichen planus have yet to be reported.

Although it is accepted that OLP is a localized disease, an increasing number of studies indicate that many significant changes in peripheral blood are implicated in the pathogenesis of OLP5, 13, 14. Several previous investigations focused on the alteration of T lymphocyte subsets in the peripheral blood of OLP patients13, however, no studies on T helper cell subsets have been reported. In the present study, we address the hypothesis that Th17,
as well as Th1, is involved in the pathogenesis of OLP. Thus, double immunofluorescence staining was performed to identify Th1 and Th17 cells in the OLP lesions. Furthermore, we detected the proportions of Th1 and Th17 cells in the peripheral blood of patients and control subjects using flow cytometry, and measured serum IFN-γ and IL-17 levels by an enzyme-linked immunosorbent assay (ELISA).

1 MATERIALS AND METHODS

1.1 Patients and Samples

A total of 40 patients with OLP, including 22 patients (8 males and 14 females with an average of 46.8 ±12.2 years old) with reticular OLP and 18 (5 males and 13 females with an average of 49.2±10.4 years old) with atrophic-erosive OLP, were enrolled in this study. The patients were diagnosed according to the clinical and histopathologic features based on the modified WHO diagnostic criteria of OLP[15]. The two subtypes were defined depending on the clinical features briefly described as following: (1) reticular OLP is the most common type which presents as a lace-like network of slightly raised gray-white lines; (2) atrophic-erosive OLP is a more severe form characterized as diffuse erythematous lesions or irregular erosive lesions with or without reticular lesions. The patients were recruited according to the inclusion and exclusion criteria described previously[16]. Patients with hypertension, diabetes, infectious diseases, allergic disorders or other autoimmune diseases, such as rheumatoid arthritis, discoid lupus erythematosus and systemic lupus erythematosus, were excluded. Furthermore, the patients included in the study had not received treatment for OLP or taken any prescription drugs for at least two months. Fifteen sex- and age-matched healthy volunteers (6 males and 9 females with an average of 43.9±11.8 years old) were recruited as controls, which had no disorders known to affect their immune function.

Peripheral blood samples were obtained from 40 patients and 15 healthy controls and placed in BD Vacutainer sodium heparin tubes and coagulant tubes (Becton Dickinson, USA), respectively. The former samples were stabilized at room temperature for flow cytometric analysis within 5 h, and the latter samples were stabilised for 1 h and centrifuged at 1000 g for 10 min to separate the serum. The serum was then transferred to clean centrifuge tubes and stored at –80°C for the measurement of IFN-γ and IL-17.

Additionally, formalin-fixed and paraffin-embedded (FFPE) tissue lesions from 16 OLP patients were utilized for immunofluorescence staining. Normal control oral mucosal samples were obtained from five healthy individuals undergoing orthognathic surgery. This study was approved by the Ethics Committee of Tongji Hospital of Huazhong University of Science and Technology (Wuhan, China), and informed consent was obtained from all subjects according to the Declaration of Helsinki with regard to research use.

1.2 Immunofluorescence

To determine Th1 (CD4+IFN-γ+ T helper cell) and Th17 (CD4+IL-17+ T helper cell) in OLP lesions, we performed double immunofluorescence staining of FFPE sections as described previously[17]. FFPE OLP tissue lesions were sectioned at 4 μm intervals and placed on coated slides. Antigen retrieval was performed by microwave heating for 20 min in 10 mmol/L citrate buffer, and blocking buffer (1% BSA in PPS) was added to the slides for 30 min at room temperature. For double staining of CD4/IFN-γ and CD4/IL-17, sections were incubated overnight at 4°C with mouse anti-human CD4 monoclonal antibody (1:100, Lab Vision, USA) and rabbit anti-human IFN-γ polyclonal antibody (1:100, Proteintech, USA) or mouse anti-human CD4 monoclonal antibody and rabbit anti-human IL-17 polyclonal antibody (1:100, Proteintech, USA). Sections were washed 3 times and incubated with Cy3-conjugated goat anti-mouse antibody and FITC-conjugated goat anti-rabbit antibody for 1 h at room temperature. Nuclei were counterstained with DAPI. Images were captured using a Nikon Eclipse Ti-S fluorescence microscope. Additionally, hematoxylin and eosin (H&E) staining of sections was used to visualize the histology of tissues. Images of H&E staining were captured using a Nikon Eclipse Ti-S microscope. Quantitative evaluation of Th1 and Th17 cells was done by analyzing five different high-power fields (200×) in each section.

1.3 Surface and Intracellular Staining, and Flow Cytometric Analysis

For intracellular cytokine staining, heparinised whole blood was diluted with the same volume of RPMI 1640 medium (Thermo Scientific HyClone, China) and incubated for 5 h in 5% CO2 at 37°C with 50 ng/mL phorbol myristate acetate (PMA) (Enzo Life Sciences, USA) and 1 μg/mL ionomycin (Enzo Life Sciences, USA) in the presence of 10 μg/mL brefedin A (Enzo Life Sciences, USA). After lysis of the red blood cells with RBC lysis buffer (Biolegend, USA), cells were washed and suspended in cell staining buffer (Biolegend, USA). Cell surface staining was performed with anti-human CD3 FITC and anti-human CD4 PerCP-Cy5.5 antibodies (eBioscience, USA) for 30 min in the dark at 4°C. For intracellular staining, cells were fixed and permeabilised with fixation buffer and permeabilisation wash buffer (Biolegend, USA), then stained with anti-human IFN-γ APC and anti-human IL-17A PE antibodies (eBioscience, USA). Isotype controls were used to exclude non-specific antibody binding. Cells were washed with permeabilisation wash buffer twice and resuspended in cell staining buffer. Flow cytometric analysis was performed on a BD LSR II Flow Cytometer (BD Biosciences, USA), and the data were processed using the FACSDiVa software (BD Biosciences, USA). The data of the flow cytometry were analyzed by using the FCS Express V4 software (De Novo Software, Canada). An initial gate based on forward scatter and side scatter properties, together with an additional CD3+CD4+ gate was defined. The proportions of IFN-γ-positive cells and IL-17-positive cells were measured in CD3+CD4+ T cells.

1.4 Enzyme-linked Immunosorbent Assay

To determine the IFN-γ and IL-17 concentration in serum, ELISA was performed using commercial ELISA kits (NeoBioscience, China) according to the manufacturer’s instructions. 100 μL of the standard, control or sample was added to each well of the microplate pre-