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Cytokine profiles in serum of patients with oral lichen planus

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ABSTRACT

Objective: Oral lichen planus (OLP) is a chronic inflammatory disorder of oral mucosa, which represents T-cell-mediated autoimmune diseases. The inflammatory response in OLP is characterized by the accumulation and expansion of T-helper 1 (Th1) lymphocytes. Several lines of evidence have suggested that a complex cytokine network plays an important role in the exacerbation and perpetuation of OLP. The aim of this study was to evaluate Th1 and T-helper 2 (Th2) cytokine profile in serum of patients with OLP in comparison to healthy controls.

Methods: Thirty patients with OLP, and 30 healthy controls participated in the study. Tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-2, IL-4, IL-5 and IL-10 levels have been measured in flow cytometry by bead based cytokine measurement.

Results: Although no statistical differences were observed in the serum levels of TNF-α, IFN-γ, IL-5 and IL-4 between OLP patients and controls ($p > 0.05$), there were statistically significant differences in the serum levels of IL-2 and IL-10 ($p < 0.05$ and $p < 0.01$, respectively). A significantly decreased tendency towards the levels of IL-2 were observed in OLP patients when compared to controls ($p < 0.05$), and the mean level of IL-10 in serum increased remarkably in the OLP patients than those in the controls ($p < 0.01$).

Conclusions: The finding of higher serum levels of IL-10 in patients in presence of low serum IL-2 levels, shows us that there is a dominance of Th2 response. This makes us think that there is a change in Th1/Th2 balance. Dominance of the Th2 response may indicate that OLP could be a result of a delayed type hypersensitivity.

1. Introduction

Oral lichen planus (OLP) is an idiopathic and relatively common disorder of the stratified squamous epithelia affecting up to 2% of the population worldwide, mainly above 40 years of age [1–4]. Clinically, it results in reticular white lesions that are usually bilaterally distributed on the oral mucosa and occasionally in the tongue. In a number of cases, these lesions are associated with areas of mucosal erosion and ulceration [1,5,6]. Histopathologically, OLP is characterized by the presence of liquefaction degeneration of basal epithelial cells and a "band-like" inflammatory cells infiltrating in the lamina propria of oral mucosa, which comprised principally with macrophages and CD4+ cells in the early stages of the lesions and predominantly CD8+ T cells in the later stages of the lesions [7,8]. The etiological factor of OLP is uncertain. However, previous studies have shown that CD4+ T cells and CD8+ T cells predominate in the lymphocytic infiltration of oral and cutaneous LP lesions. These T cells produce and respond to a range of cytokines, such as tumor necrotizing factor-alpha (TNF-α), which have an important role in the initiation and progression of OLP and other inflammatory mediators [9].

Evidence from investigations using mouse and human models has indicated that T helper cells can be classified into at least two functional subsets, Th1 and Th2, on the basis of their cytokine profiles. Th1 cells are characterized by the production of IL-2 and IFN-γ, and are critical in the cell-mediated immunity, but Th2 cells are characterized by the production of IL-4, IL-5, and IL-10 and play an important role in humoral immunity. These two Th subsets regulate each other's function through the antagonistic activity of their respective cytokines which determine in large extent the characteristics of immune responses and developments [10–12]. Many studies have demonstrated a close relationship between Th1/Th2 imbalance and pathogenesis of a series of autoimmune disorders including Behçet's disease (Th1 dominant response), Sjögren syndrome secondary to hepatitis C virus infection (Th2 dominant response) and psoriasis (Th1 dominant response) [10,13]. Increasing evidences showed the balance of immune responses between Th1 and Th2 determined outcomes. These studies have described the expression pattern of the different cytokines/

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chemokines, which involved in Th1 and/or Th2 polarization, in tissues, cultured cells, and serum from patients with OLP [10,14,15]. In these studies, Sugerman et al. [5] and Khan et al. [3] observed that OLP was characterized by Th1 cytokine bias. In contrast, Rhodus et al. [10] indicated the possibility that a Th2-dominated immune response does occur in a subgroup of OLP patients.

These results indicate that the different patterns of Th1/Th2 imbalance, Th1 or Th2 overactivation or mixed Th1/Th2 conditions, may occur in the pathogenesis of OLP. Therefore, in this study we aim to evaluate Th1 and Th2 cytokine profile in serum of patients with OLP.

2. Patients and methods

2.1. Selection of study group

Thirty patients with OLP and 30 age-gender matched healthy controls were participated in the study. The diagnosis of OLP was made with biopsy confirmation in agreement with the patient's clinical features and conventional histopathological diagnosis was confirmed according to the World Health Organization's clinicopathological diagnostic criteria for OLP [16]. A detailed medical history of each patient was taken. All patients with OLP were free from any systemic disease and did not receive any medication either topical or systemic that could cause lichenoid reaction during the 3 months before the study. Moreover, patients with suspected restoration-related reaction or gingival inflammation were excluded from this study.

Thirty controls composed of faculty personnel were healthy normal individuals free from any systemic diseases or inflammatory oral lesions, participated after giving informed consent. The OLP patients were also requested to sign a written informed consent statement. The study was carried out according to the recommendations of the Helsinki declaration and the study protocol was approved by the Local Committee of Research and Ethics of Marmara University.

2.2. Collection of blood samples

Blood samples were collected by a phelobotomist into red top Vacutainer tubes (BDBioSciences, USA). Consent forms were checked by the phlebotomist before each collection. Tubes were kept at room temperature for one hour and allowed to clot, serum was transferred to cryotubes and kept at −20 °C until the day of the analysis for 3 months.

2.3. Measurement of cytokine production

Cytokine measurements were performed with BD Cytometric Bead Array Th1/Th2 Human kit (BDBioSciences, USA). This kit was able to measure serum Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-5 (IL-5), Interleukin-10 (IL-10), Tumor Necrotizing Factor-alpha (TNF-α) and Interferon-gamma (IFN-γ) levels, simultaneously. All of the serum samples were used after thawing and centrifugation. Standard curves were obtained by using reference standard beads. Quantitative results were evaluated with BD FCAP program (BD BioSciences, USA). All of the results were also calculated manually to check the results obtained with FCAP software (Fig. 1).

2.4. Statistical analysis

The data were analysed with NCSS (Number Cruncher Statistical System) 2007 and PASS 2008 Statistical Software (Utah, USA). Descriptive statistical methods (mean, standard deviation, frequency) were used for the evaluation of the data. The gender matching between patients and controls was analysed by means of the chi-square test. Statistical significance of differences among the groups was determined by Student t test (for parametric distribution), Kruskal Wallis test and Mann Whitney U test (for non-parametric distribution), and to calculate effect size for each pair of groups that differs a significantly in Mann–Whitney follow-up (Post Hoc Mann Whitney U test). *P* values of less than 0.05 were interpreted as significant, and the level in confidence intervals was 95%.

3. Results

3.1. Characteristics of the study group

Thirty patients diagnosed with OLP and 30 matched healthy controls were studied. The mean ages of OLP patients and controls were 51.10 ± 12.25 and 48.09 ± 11.92, respectively. Of the total 30 cases of OLP identified 21 (70.0%) were female and 9 (30.0%) were male, and of the 30 controls 18 (60.0%) were female and 12 (40.0%) were male. The two groups did not differ for age and gender ($p > 0.05$). The distribution of subjects according to the age and gender is presented in Table 1 and characteristics of patients with OLP were listed in Table 2.

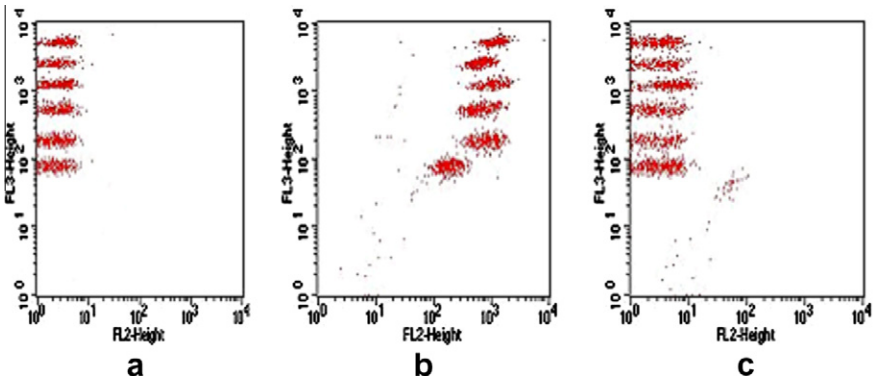

Fig. 1. CBA assay scattergrams. BD CBA assays provide a method of capturing a soluble analyze or set of analyzes with beads of known size and fluorescence, making it possible to detect analyzes using flow cytometry. Each capture bead in the kit has been conjugated with a specific antibody. The detection reagent provided in the kit is a mixture of phycoerythrin (PE)-conjugated antibodies, which provides a fluorescent signal in proportion to the amount of bound analyze. The six bead populations are mixed together to form the bead array, which is resolved in a red channel (FL3) of a flow cytometer. By scaling red channel (FL3) across PE measuring FL2 it is possible to discriminate different levels of cytokines. A negative control (a), a positive control (b) and an example of patient (c), respectively.