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Original Article

Frequencies of abnormal humoral and cellular immune component levels in peripheral blood of patients with recurrent aphthous ulceration

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Abstract *Background/purpose:* Recurrent aphthous ulceration (RAU) has an incidence of approximately 20% in general population. However, its exact cause remains unknown. Increasing evidence suggests that immunologic mechanisms may play crucial roles in the etiology of this disease.
Materials and methods: The peripheral blood samples were obtained from 85 patients with RAU during acute phase and 87 healthy controls. The serum levels of IgG, IgA, IgM, C3 and C4 were measured by immunoturbidimetry. In addition, the serum IgE levels were measured by electro-chemiluminescence immunoassay. Furthermore, the percentages of B, T, $CD4^+$ T, $CD8^+$ T lymphocytes and natural killer (NK) cells in peripheral blood were determined by flow cytometry.
Results: Our findings showed that the serum IgG, IgA, IgE, C3 and C4 levels of RAU patients were significantly higher than those of healthy controls. The percentages of $CD4^+$ T cells and B cells in peripheral blood of RAU patients were significantly decreased, whereas the

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percentages of $CD8^+$ T cells and NK cells of RAU patients were remarkably increased. Our results indicated that the IgG level was elevated in 18 patients (21.2%) and that the IgE level was increased in 21 patients (24.7%). Our results also showed that the frequency of abnormal IgG or IgE levels were significantly correlated with that of abnormal $CD8^+$ T cell percentage in RAU patients.

Conclusion: The levels of both humoral and cellular immune components could be altered in RAU. The relationship between humoral and cellular immune may be potentially important immunologic aspects involved in the pathogenesis of RAU.

Introduction

Recurrent aphthous ulceration (RAU) is an inflammatory disease with an incidence of approximately 20% in general population. The etiology of RAU is multifactorial, including genetic, immune, nutritional, and microbial factors and local trauma. Immune mechanisms appear to play important roles in the pathogenesis of RAU.[1–14]

In terms of humoral immunity, some studies have confirmed that immunoglobulin levels were altered in RAU patients.[2–6] The serum IgA level was elevated in minor RAU patients compared with healthy controls.[2] In addition, the levels of both salivary IgG subclasses and IgA2 were increased in RAU patients.[3] Moreover, an elevated serum IgE level has been detected in RAU patients in association with disease characteristics.[6] In addition, a study has confirmed that serum C3 and C4 levels were higher in RAU than that in healthy control.[7]

Cellular immunity was proven to play a crucial role in the pathogenesis of RAU. Diminished percentages of total T cells, $CD4^+$ T cells and B cells have been observed in the peripheral blood of RAU patients, as well as increased percentages of $CD8^+$ T cells and natural killer (NK) cells and a reduced $CD4^+/CD8^+$ ratio.[8–14]

Although previous studies have focused on the abnormality of humoral or cellular immunity in RAU, to the best of our knowledge, the relationship between humoral and cellular immunity in this disease has not yet been evaluated. In the present study, we not only assessed the alterations in humoral and cellular immunity in RAU patients, but also attempted to explore the correlations between humoral and cellular immunity of these patients.

Materials and methods

Study subjects

This study was approved by the Ethics Committee of the Nanjing Stomatological Hospital, Medical School of Nanjing University (IRB Approval Number: 2014NL-002 (ks)). A total of 85 RAU patients during the acute phase were included in this study. 87 age- and sex-matched healthy volunteers with no history of any episodes of RAU were also included in the present study. All of the subjects were recruited between 2015 and 2017, and written informed consent was obtained from each participant. The 85 RAU patients had a history of regularly recurring oral ulcers and had experienced at least three episodes in the last 6 months. For all the patients included, the clinical diagnosis was classified as minor according to the standard classification of RAU. The clinical characteristics of oral ulcer were recorded in detail. Through measuring the semi-major and semi-minor axis sizes (a and b) (cm) of one oral ulcer, we calculated the area of single ulcer according to the formula of oval area: $S = \pi ab$. Then we determined the area of ulcers (cm^2) in one patient with RAU by calculating total areas of all oral ulcers. Peripheral blood samples were obtained from healthy controls and all patients within 48 h after the development of aphthae. The exclusion criteria included diagnosis of an autoimmune disease (e.g., systemic lupus erythematous, rheumatoid arthritis, psoriasis, or diabetes), any type of cancer, HIV, hepatitis B or C infection, any hematologic deficiencies, other oral mucosal diseases (e.g., oral lichen planus, pemphigus), systemic disorders (e.g., Behçet's disease, Crohn disease), chronic medication use, any mental disorder, women of pregnancy or lactation. Subjects were considered eligible if they had not taken any medication for 3 months prior to examination. All the patients and healthy controls included in the present study were non-smokers.

Immunoturbidimetry

Peripheral blood samples (5 ml) were collected from the patients by venipuncture between 8 and 10 a.m. The samples were transported on ice in an upright position to the lab within 2 h. The serum samples were obtained by centrifugation and then stored at −80 °C until use. Assays for IgG, IgA, IgM, and the complements C3 and C4 were performed using immunoturbidimetry methods. In addition, the serum IgG, IgA, and IgM levels were measured using commercial kits (Sichuan Marker Biotechnology, Chengdu, China) with an automated analyzer (Roche Diagnostics, IN, Switzerland) following the manufacturer's instructions.

Electro-chemiluminescence immunoassay

Serum IgE levels were measured using electro-chemiluminescence immunoassay kits (Roche Diagnostics GmbH, Mannheim, Germany) with a Cobas e 601 system (Elecsys module) immunoassay analyzer (Roche