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Original Article

# Detection of the peripheral blood antigens and clinical value in recurrent aphthous ulcer: A cross-section study

Dan Pan [a,1], Mao-feng Qing [a,1], Dan-hua Ma [a], Jiao Wei [a,b], Zhen Wang [a], Lu-yao Cai [a], Hao Xu [a**], Yu Zhou [a*], Qian-ming Chen [a]

[a] State Key Laboratory of Oral Diseases, National Clinical Research Center for Oral Diseases, Chinese Academy of Medical Sciences Research Unit of Oral Carcinogenesis and Management, West China Hospital of Stomatology, Sichuan University, Chengdu, Sichuan, China

[b] Department of Oral Mucosal Diseases, State Key Laboratory of Military Stomatology and National Clinical Research Center for Oral Disease and Shaanxi Key Laboratory of Stomatology, School of Stomatology, The Fourth Military University, Xi'an, Shanxi, China

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Recurrent aphthous ulcer;
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*Abstract Background/purpose:* Recurrent aphthous ulcer (RAU) is one of the most common diseases of oral mucosa, which is generally believed to be related to immunity, though the etiology is still unclear. It is suspected that allergies are directly related to RAU. So we sought to explore the relationship between hypersensitivity and RAU.

*Materials and methods:* 40 RAU patients who were in ulceration period and 40 people who were in good health were selected from Jan 2016 to Feb 2017, matched in age and sex. The peripheral blood antigens of 40 RAU patients and 40 healthy people was tested, and serum specific IgE (sIgE) with 6 groups of antigens and total IgE (tIgE) analysis was performed to identify IgE-mediated allergic reaction possibly affecting RAU. We then investigated the food intolerance and IgG levels to discover the correlation between non-IgE mediated allergic reaction and RAU.

*Results:* The positive cases and rate of sIgE in RAU group was higher than that of control, but the difference was not statistically significant ($P > 0.05$). Positive grade of animal fur scraps

* Corresponding author. State Key Laboratory of Oral Diseases, National Clinical Research Center for Oral Diseases, Chinese Academy of Medical Sciences Research Unit of Oral Carcinogenesis and Management, West China Hospital of Stomatology, Sichuan University, No. 14, Sec 3 Renminnan Road, Chengdu, Sichuan, 610041, China.

** Corresponding author. State Key Laboratory of Oral Diseases, National Clinical Research Center for Oral Diseases, Chinese Academy of Medical Sciences Research Unit of Oral Carcinogenesis and Management, West China Hospital of Stomatology, Sichuan University, No. 14, Sec 3 Renminnan Road, Chengdu, Sichuan, 610041, China.

*E-mail addresses:* hao.xu@scu.edu.cn (H. Xu), 812471898@qq.com (Y. Zhou).

[1] These two authors contributed equally to this work.

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(EX1), house dust mixed (HX2) and the serum tIgE concentration of the RAU group were significantly higher than the control group ($P < 0.05$).The number of food intolerance in RAU group was significantly higher than that in control group ($P < 0.05$).
*Conclusion:* Our findings suggested certain correlation between RAU and anaphylaxis. Daily contact allergens and food intolerance may be one of the causes of RAU. Moreover, this provides reference value for clinical diagnosis and treatment.


## Introduction

Recurrent aphthous ulcer (RAU) is also known as recurrent aphthous stomatitis (RAS) or recurrent oral ulcer (ROU), which is the most common oral mucosal disease.[1] Generally, 10%—25% of population suffered from the disease, especially at the age of 10—30, and female are more susceptible.[2—4] The disease is characterized by mucosal recurrent ulcer with periodicity, self-limited and burning pain. There are three main clinical subtypes: major, minor, and herpetiform RAU.[3] Patients suffer pain when swallowing, eating and speaking, which impact the quality of their lives.[5]

The etiology and mechanism of RAU has not yet been fully clarified so far.[4] Most researchers believed that it is the result of a combination of factors such as immunity, genetics, systemic diseases, infections, etc.[6] In recent years, a large number of studies have suggested that immunity is the most important pathogenesis of RAU, and the formation of cellular immunity and immune complex leads to immune disorders to participate in the pathogenesis, especially the cellular immune response is related to the occurrence of RAU.[7]

Hypersensitivity is defined as an immune-mediated allergic reaction initiated primarily by an IgE dependent immune response to antigens.[8] In many previous literatures, food hypersensitivity can initiate and aggravate the clinical manifestation of RAU.[9,10] But the relationship between RAU and hypersensitivity is not clear, and its pathogenic mechanism, clinical features and the process of are partially overlapped with hypersensitivity, with the participation of T cells and cytotoxicity reaction, all of which are recurring, reversible and intermittent.[11—14] Both RAU and hypersensitivity reactions usually relieve after each attack, with a period of interval between episodes. And recur within days, moths, or years.

IgE plays an important role in the occurrence and development of hypersensitivity related diseases.[15] Serum specific-IgE detection test has an important guiding significance for the diagnosis of hypersensitivity related diseases and the identification of allergic reactions.[16,17]

The Uni-CAP detection system is the most representative of the detection methods for detecting serum sIgE antibodies in the current peripheral blood allergen detection methods. In our preliminary work, we included 13 articles from 6 databases including PubMed to evaluate the sensitivity of skin prick test and Uni-CAP (Supplementary Table 1). We found that the sensitivity, specificity and accuracy of the method of detecting antibody in serum by Uni-CAP detection system were better, and it could be used as a more reliable allergen diagnosis method.

Food hypersensitivity is always divided into allergic and non-allergic food hypersensitivity, and the latter one is also called food intolerance.[18,19] The measurement of food-specific IgG can reflect the tolerance of the host to food.[19] In previous studies, little evidence of food allergy in most patients with RAU.[20,21] Therefore, in this study, we used the detection of IgG to determine whether RAU patients have more food intolerance.

In this study, we utilized Uni-CAP system to detect serum tIgE and sIgE antibodies in RAU patients, and detect the food allergen IgG antibodies by Allerquant IgG Food Allergy Screening ELISA Kit. We sought to study the relationship between RAU and hypersensitivity reaction, and provide basis for elucidating the pathogenesis of RAU and preventing recurrence.

## Materials and methods

### Ethics approval

All procedures adopted in this study were approved by Ethics Committee of West China Hospital of Stomatology, Sichuan University (WCHSIRB-D-2015-162).

### Samples

The study was performed in a single academic hospital, West China Hospital of Stomatology, Sichuan University, Chengdu, China. 40 RAU patients who were in ulceration period and 40 people who were in good health were selected from Jan 2016 to Feb 2017, matched in age and sex.

The inclusion criteria were:(1) clinic manifestations met the dignosis of RAU. (2) The ulcers appeared within 3 days. (3) The history of RAU was over 1 year.

The exclusion criteria were: (1) Other diseases in relation to oral ulcers. (2) The medication history (glucocorticoid and antihistamines history) in recent 4 weeks. (3) Pregnancy or systemic diseases. (4) The history of tobacco and alcohol use.

### Total IgE and special IgE

1 ml of Blood for every patient were allowed to clot at room temperature for 20—30 min, centrifuged at 3000r for 10 min at 4 °C, separated and stored in aliquots at 80 °C until analysis.

All supernatants and sera were assayed for an IgE screening for inhalant allergens (Phadiatop), total and specific IgE by the Uni-CAP system (Pharmacia & Upjohn, Upsala, Sweden). The automatic immunofluorescence analyzer will output the test results to the Information Data Manager software.

### Food-specific IgG

14 kinds of food-specific IgG kits produced by BIOMERICA company (Biomerica, Inc. Newport Beach, CA, USA) were used to detect the food-specific IgG antibody. And the experiment was conducted strictly according to the instructions of the kit. Firstly, we prepared the standard serum of 50U/ml, 100U/ml, 200U/ml, 400U/ml respectively and drew a standard curve. Then, the sample serum to be detected was diluted with a ratio of 1: 100. After incubation at room temperature (22—25 °C) for 1 h, each reaction pore was cleaned with cleaning liquid; each reaction pore was cleaned with cleaning solution after adding 100 μl anti-human IgG antibody-horseradish peroxidase binding solution into all reaction poresn and incubation at room temperature 22—25 °C for 30 min; after incubation with 100 μl working substrate buffer mixture (equal proportion mixture of substrate buffer A and substrate buffer B) in all the pores at room temperature 22—25 °C for 10 min, 50 μl stop buffer was added into all reaction pores.

The Varioskan Flash full-wavelength multi-functional enzyme labeling facility manufactured by Thermo Fisher (Thermo Fisher Scientific,Inc. Waltham, MA, USA) was used to measure absorbance values at 450 nm per pore.

### Data manager software

The antibody content of sIgE <0.35 kU/l was negative. The antibody content of sIgE >0.35 kU/l was positive. Divided into 6 positive grades. 0.35 kU/l～0.7 kU/l was grade1, 0.7 kU/l～3.5 kU/l was grade 2, 3.5KU/l～17.5 kU/l was grade 3, and 17.5 kU/l～50 kU/l was grade 4, and 50kU/l～100 kU/l was grade 5, >100KU/l was grade 6.

### Standard curve and outcome evaluation

According to the experimental results, we could use the optical density (O.D.) of a blank control pore, standard pore of 50U/ml, standard pore of 100U/ml, standard pore of 200U/ml and standard pore of 400U/ml to draw the standard curve.

When the level of food-specific IgG of sample was less than 50U/ml, it was negative; When the level of food-specific IgG was more than 50U/ml, it was positive: when the level of food-specific IgG was more than 50U/ml and less than 100U/ml, it was weakly positive (+); when the level of food-specific IgG was more than 100U/ml and less than 200U/ml, it was positive (++); when level of food-specific IgG was more than 200U/ml, it was strongly positive (+++).

### Data analysis

The positive rate of each sIgE and total serum sIgE and number of positive cases (positive rate) of total food intolerance of 14 kinds of foods between RAU group and control group were compared by $\chi 2$ test. Comparison of tIgE content between RAU group and control group was carried out by variance homogeneity test and t test. All the data of this experiment were analyzed by software SPSS 22.0. Significant level $\alpha = 0.05$.

## Results

### Total IgE levels between RAU and controls

There were 9 positive cases for serum sIgE in the RAU group, and the positive rate was 22.50%; while positive cases in the control group were 3, and the positive rate was 7.50%. Using the X2 test to compare the data of two groups, the results show that the positive rate of sIgE in RAU group was higher than that in control group, but the difference between them were not statistically significant ($P > 0.05$) (Table 1).

### IgE-antibodies to 6 groups of allergens between RAU and control

Next, we tested and compared the amount of serums sIgE levels of animal fur scraps (EX1), house dust mixed (HX2), shrimp (F24), food (FX5), mold (MX2) and weed pollen (WX5) in RAU and controls. There were 4 positive cases in EX1 (10%), 2 positive cases in FX5 (5%), 6 positive cases in HX2 (15%), and 1 in MX2 (2.5%), 2 in WX5 (5%), 6 in F24 (15%) respectively. And in the control group, equivalently, there were 2 positive cases in MX2 (5%), only 1 positive case in both HX2 (2.5%) and F24 (2.5%), while the detection results of EX1, FX5 and WX5 were negative (Table 2).

The EX1, HX2, F24, FX5, MX2, WX5 were analyzed by chi-square test and no significant difference between patients with RAU and the healthy people ($P > 0.05$) was detected.

### Positive grade of the 6 kinds of sIgE between RAU and control

In RAU group, there were two, one, one, one grade 1 positive cases respectively in EX1, FX5, HX2, MX2; there were two, one, two, two, three grade 2 positive cases respectively in EX1, FX5, HX2, WX5, F24; there were two grade 3 positive cases in HX2; in addition, the only two grade 4 cases belong to HX2 and F24, respectively. In the control group, there was only one grade 1 positive case in F24; there were one, two, one grade 2 positive cases respectively in HX2, MX2 and F24. And the detections of others

**Table 1** Comparison of positive cases of total serum specific IgE between RAU[a] group and control group.

| Group | positive cases | negative cases | total | positive rate (%) |
|---|---|---|---|---|
| RAU[a] | 9 | 31 | 40 | 22.5 |
| Control | 3 | 37 | 40 | 7.5 |
| Total | 12 | 68 | 80 | 15 |

[a] RAU: recurrent aphthous ulcer.

**Table 2** Comparison of positive cases (positive rate) of 6 kinds of allergens for serums sIgE between RAU group and control group.

| Groups (RUA/C) Positive Cases/ grade | EX1[a] | FX5[b] | HX2[c] | MX2[d] | WX5[e] | F24[f] |
|---|---|---|---|---|---|---|
| positive cases | 4/0 | 2/0 | 6/0 | 1/2 | 2/0 | 6/2 |
| grade 1 | 2/0 | 1/0 | 1/0 | 1/0 | 0/0 | 2/1 |
| grade 2 | 2/0 | 1/0 | 2/1 | 0/2 | 2/0 | 3/1 |
| grade 3 | 0/0 | 0/0 | 2/0 | 0/0 | 0/0 | 0/0 |
| grade 4 | 0/0 | 0/0 | 1/0 | 0/0 | 0/0 | 1/0 |
| Z | −2.038 | −1.423 | −1.983 | −0.614 | −1.423 | −1.507 |
| *P* | 0.042[a] | 0.155[b] | 0.047[a] | 0.539[b] | 0.155[b] | 0.132[b] |

[a] The amount of serums sIgE levels in animal fur scraps.
[b] The amount of serums sIgE levels in food.
[c] The amount of serums sIgE levels in house dust mixed.
[d] The amount of serums sIgE levels in mold.
[e] The amount of serums sIgE levels in weed pollen.
[f] The amount of serums sIgE levels in shrimp.

allergens were all 0 (Table 2). After compared using Rank Sum Test, the results showed that the positive grades of sIgE in EX1, FX5, HX2, WX5 and F24 were higher than control group, but only EX1 and HX2 had significant statistic difference ($P < 0.05$). And the sIgE positive grade of MX2 in RAU group was lower than control group, but the differences were not statistically significant ($P > 0.05$) (Table 2).

### Serum tIgE level between RAU group and control group

Comparison of tIgE content between RAU group and control group was carried out by variance homogeneity test and t test. The serum tIgE concentration of the RAU group was significantly higher than the control group, and the difference was statistically significant ($P < 0.05$) (Table 3).

### IgG-antibodies to 14 kinds of food

To explore the relationship between food intolerance and RAU, we tested for food specific IgG with 14 kinds of foods and analyzed the data by X2 test ($P < 0.05$). In RAU group, 32/40 (80%) cases were positive which represented a significant increase over the control group (50%) (Table 4).

**Table 3** Analysis of content of serum total IgE (tIgE) between RAU[a] group and control group.

| Group | cases | content of serum tIgE |
|---|---|---|
| | | Mean S.d |
| RAU | 40 | 129.8758 243.3124 |
| Control | 40 | 47.1465 7.4028 |

[a] Recurrent aphthous ulcer.

Next, we analyzed the number of types of food intolerance between RAU group and control group. In RAU group, 12 were intolerant of 1 kind of food, 6 cases were intolerant of 2 kinds of food, 3 cases were intolerant of 3 kinds of food and 10 cases were intolerant of 4 or more kinds of food. And in the control group, 11 cases were intolerant of 1 kind of food, 8 cases were intolerant of 2 kinds of food, 1 case were intolerant of 3 kinds of food and none were intolerant of 4 or more kinds of food. The number of food intolerance in RAU group was significantly higher than that in control group by rank sum test ($P < 0.05$) (Table 4).

We also analyzed the degree of intolerance to each kind of food in RAU group and control group. Comparing the degree of intolerance to each kind of food, the results showed that the intolerant degree of 10 kinds of food like cod, corn, crab, egg, white/egg yolk, milk, rice, shrimp, soybean, tomato and wheat in the RAU group was higher than that in the control group (Supplementary Table 2), of which milk, rice, shrimp and soybean was significant difference between the RAU group and the control group ($P < 0.05$). But there was no significant difference of intolerance to the other 6 kinds of foods between the two groups ($P > 0.05$). The intolerant degree of beef in the RAU group was same with the control group; The intolerant degree of chicken, mushroom, milk and pork in the RAU group was lower than that in the control group, but no significance difference ($P > 0.05$).

## Discussion

As one of the most common oral diseases, RAU has a high incidence and has a great impact on the quality of life of patients when it occurs. However, the etiology of RAU is unknown at present, and allergy may be related to it. Our study is to try to understand the relationship between them.

In the study, we found the concentration of serum tIgE antibody in RAU group was significantly higher than in the control group, which may imply the abnormal immune response in RAU patients. The results were similar to some of previous studies that some antigen-specific mechanisms may be engaged in the pathogenesis of RAU.[9,10,22] Furthermore, we believe that one of the intriguing

**Table 4** Comparison of positive cases and level of food intolerance between RAU[b] group and control group.

| Characteristics of food intolerance | RAU | Control | $\chi 2/Z$ | *P* |
|---|---|---|---|---|
| Classify of food intolerance | | | $\chi 2 = 7.912$ | 0.005[a] |
| Positive | 32 | 20 | | |
| Negative | 8 | 20 | | |
| Level of food intolerance | | | $Z = -3.540$ | <0.001[a] |
| 1 | 12 | 11 | | |
| 2 | 6 | 8 | | |
| 3 | 4 | 1 | | |
| ≥4 | 10 | 0 | | |

[a] $P < 0.05$, the difference is statistically significant.
[b] Recurrent aphthous ulcer.

findings of our study was the first time to explore the association between RAU and specific allergen. RAU group matched higher positive grades in the six allergens. Among them, the difference in EX1 and HX2 are statistically significant, which are all daily contact allergens. Whereas, there were no significant differences in dietary allergens previously associated with RAU, such as food and shrimp. RAU and IgE-mediated type I allergens may have a certain correlation, but their correlation needs further research.

In addition to allergic food allergies, food intolerance is also an important reason of food hypersensitivity.[23,24] Food intolerance are adverse immunological responses to food proteins.[25] For example, lactose intolerance is high frequently in Asians.[26,27] We detected the specific serum IgG of 14 kinds of food in the RAU group and control group. It was found that the positive rate of total food intolerance to 14 kinds of foods was 80.00% in RAU group and 50.00% in the control group. The positive rate of total food intolerance to 14 kinds of foods and the number of food intolerance in RAU group were significantly higher than the control group. The intolerant degree of milk, rice, shrimp and soybean in the RAU group was statistically significant compared with the control group ($P < 0.05$). Food intolerance is regarded as the lack of relevant enzymes, which causes food to become foreign bodies in the lymphoid tissue of the gastrointestinal mucosa to lead to type II/III hypersensitivity.[28] And food intolerance is considered associated with some immune-related digestive tract diseases as irritable bowel syndrome and Crohn's disease.[29,30] RAU is closely related to the body's immunity and also closely related to digestive tract diseases.[24,31,32] In our study, food intolerance was considered related with RAU. RAU group had more total positive cases and rate of food intolerance, and in milk, rice, shrimp and soybean, which are often appearing in daily life. So the another one of interesting findings of our study was that food allergy maybe not the cause of RAU, but food intolerance may be one of the causes of RAU.

In summary, we have demonstrated that RAU is associated with hypersensitivity and food intolerance. Daily contact allergens and food intolerance may be part of the causes of RAU. Avoiding contact with allergens and necessary desensitization treatment may be helpful for the recovery of RAU. Further research needs to expand the sample size to clarify the relationship between RAU and anaphylaxis and explore the mechanism of the development of RAU caused by anaphylaxis.

## Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2022.05.014.

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