

Serum Autoantibodies to Desmogleins 1 and 3 in Patients with Oral Lichen Planus

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Aim To determine the presence of circulating autoantibodies to desmoglein (Dsg) 1 and Dsg 3 in patients with oral lichen planus.

Methods Serum concentrations of circulating autoantibodies to Dsg 1 and Dsg 3 were determined by ELISA in 32 patients with erosive form and 25 patients with reticular form of oral lichen planus, 13 patients with acute recurrent aphthous ulcerations and 50 healthy controls. Indirect immunofluorescence analysis was also performed.

Results Concentrations of circulating autoantibodies to both Dsg 1 and Dsg 3 detected in the sera of patients with erosive form of oral lichen planus were significantly increased in comparison with those in healthy controls, patients with recurrent aphthous ulceration, and those with reticular oral lichen planus ($P < 0.001$ for both anti-Dsg autoantibodies). Indirect immunofluorescence also revealed significantly more positive findings in patients with erosive oral lichen planus (18 positive of 22 tested) than in healthy controls (1 positive of 20 tested; $P < 0.001$), patients with recurrent aphthous ulceration (1 positive of 10 tested; $P < 0.001$), and those with reticular oral lichen planus (3 positive of 15 tested; $P < 0.001$).

Conclusion Humoral autoimmunity seems to be involved in the pathogenesis of oral lichen planus. The differences in the serum concentration of desmoglein autoantibodies suggested that pathological mechanisms in erosive and reticular forms of oral lichen planus might not be the same.

Oral lichen planus, a chronic disease and one of the most common dermatoses of the oral mucosa, is characterized by white streaks in a lace-like pattern on the tongue and/or buccal mucosa. The disease is also accompanied by chronic inflammation the degree of which correlates with the intensity of the symptoms. The disease has several forms: atrophic, erosive, reticular, and bullous, with erosive form being the predominant initial presentation (1). The etiology of oral lichen planus is still poorly understood, but the disease is considered to be autoimmune (2,3). The precipitating factors can be stress, particular food, dental plaques, systemic illness, and poor oral hygiene (2). There is substantial evidence that the pathogenesis of oral lichen planus involves a T-cell mediated process directed against basal keratinocytes (3-5), but no oral lichen planus-specific antigen has yet been identified. There is some evidence that humoral immunity may also be involved. Immunoglobulins, fibrinogen, and C3 complement may be present in the basement membrane within lesional and perilesional tissue (6), and levels of salivary IgG and IgA subclasses may also be altered (7). The presence of circulating antibodies to a lichen planus-specific antigen on the granular and deep epithelial prickle cells in the skin lesions of lichen planus was suggested (8), although the antigen itself was only infrequently demonstrated (9,10). The presence of antiepithelial antibodies was reported in patients with oral and cutaneous lichen planus associated with drug therapy, but the antibodies were generally present only in low concentrations (11). Ingafou et al (12) reported that oral lichen was not associated with IgG circulating antibodies to epithelial antigens. However, we have previously indicated the possibility of the presence of circulating antibodies to desmoglein 1 and desmoglein 3 in patients with oral lichen planus (13). Autoantibodies to desmoglein 1 and desmoglein 3, desmosomal cadherins expressed in stratified squamous epithelia and involved in cell-to-cell adhesion (14), play a pathogenic role

in autoimmune bullous diseases, causing disruption of desmosoms and consequent acantholysis (15). Commercial ELISA tests for desmoglein 1 and desmoglein 3 autoantibodies are now available and have been proposed as a routine diagnostic tool (16). Although oral lichen planus and recurrent aphthous ulceration manifest disparate clinical appearances and natural history, both of these oral mucosal diseases seem to share immunopathological features that involve T-cell mediated response to an antigenic stimulus in the epithelium (17,18).

Our aim was to determine the presence of circulating autoantibodies to desmoglein 1 and desmoglein 3 in a group of patients with oral lichen planus and to compare them with healthy controls and patients with recurrent aphthous ulceration.

Patients and methods

Patients

The study included 57 patients with oral lichen planus who were treated at the Department of Oral Medicine, Zagreb University School of Dental Medicine, from January 2001 to October 2004 (Table 1). The diagnosis of oral lichen planus was confirmed histopathologically. To exclude patients with other similar diseases, such as pemphigus, mucous membrane pemphigoid, erythema multiforme, and lupus erythematosus, direct and indirect immunofluorescence methods were applied. The presence of subepithelial deposits of fibrinogen and related substances by anti-fibrinogen antisera differentiated oral lichen planus from all other diseases except for lupus erythematosus. The diagnosis of lupus erythematosus was excluded by finding of globular pattern of subepithelial fibrin deposit in the absence of positive immunofluorescence with anti-immunoglobulin and anti-C1 antisera. Twenty-one of 57 patients with oral lichen planus were receiving the following medications: non-steroidal anti-inflammatory drugs (1 patient), other analgesics (3

patients), antibiotics (2 patients), β -blockers and calcium channel blockers (6 patients), and other medications (15 patients). None had received immunosuppressive therapy 6 months before testing.

Patients with recurrent aphthous ulceration were diagnosed according to the clinical criteria for aphthae differentiation according to Lehner (19). Eight patients had minor and 5 had major recurrent aphthous ulceration. None of the patients suffered from any systemic disease and none received any systemic or topical therapy that could influence the results of tests performed in this study.

A group of healthy controls consisted of 50 apparently healthy volunteers.

Informed consent according to Helsinki Declaration II was obtained from each subject before the study (20), and the study was approved by the Ethics Committee of the Zagreb University School of Dental Medicine.

Methods

Peripheral blood for determination of circulating autoantibodies was drawn from each participant between 8 AM and 9 AM. Serum was separated and stored in several samples at -20°C until use. Repeated freeze-thawing was avoided.

Determination of desmoglein autoantibodies

Commercial enzyme-linked immunosorbent assay (ELISA, Medical and Biological Laboratories Co. Ltd, Nagoya, Japan) was used to determine autoantibodies to both desmogleins 1 and 3 (MESACUP Desmoglein test [Dsg 1], Code No. 7680E, and [Dsg 3], Code No. 7685E). According to the manufacturer's instructions, sera were diluted 100 \times and tested in duplicate. Desmoglein autoantibodies were determined by horseradish peroxidase-conjugated antihuman IgG with tetra-methylbenzidine as the substrate. The optical densities were read by the Dynatech MRX Microplate Reader at 450 nm, with 650 nm reference filter. The results were presented

in units per milliliter of sera (U/mL). The assay range was 5-150 U/mL, and reproducibility (coefficient of variability) was <15%.

Indirect immunofluorescence assay

Standard indirect immunofluorescence with monkey esophagus (indirect immunofluorescence IIFT Epidermis-esophagus, Code No. FA 1501-1005, Euroimmun, Lubeck, Germany) and rat bladder (IIFT Transitional epithelium, Code No. FA 1507-1005, Euroimmun) as substrates was carried out to determine autoantibodies to epithelial antigens in subject's sera. Fluorescein isothiocyanate-conjugated goat anti-human IgG specific for gamma chains was used as the second antibody. Patient sera were screened at a dilution of 1:20 in phosphate-buffered saline. All slides were assessed by two well-trained observers in a blinded fashion.

Statistical analysis

As the data did not show normal distribution, the results were presented as medians with interquartile ranges and analyzed by Kruskal-Wallis test. Data on anti-desmoglein autoantibodies were analyzed with Mann-Whitney U test, and data obtained by indirect immunofluorescence were analyzed with χ^2 test. On the basis of the observed differences, variability of the results, and group sample sizes, the comparisons achieved power between 0.78 and 0.99, with a significance level set at 0.05 using a two-sided Mann-Whitney tests and assuming that the actual distributions were double exponential. The statistical package used was Statistics with graphics for Macintosh Version 1.0 (Data Metrics Inc., Philadelphia, PA, USA, 1985). P values <0.05 were considered statistically significant.

Results

Concentrations of both anti-desmoglein 1 and anti-desmoglein 3 autoantibodies in the patients with recurrent aphthous ulcerations and those with reticular form of oral lichen planus

Table 1. Characteristics, serum concentrations of autoantibodies to desmoglein 1 and 3, and indirect immunofluorescence findings in 57 patients with oral lichen planus or recurrent aphthous ulceration, and healthy controls

| Characteristic* | No. of patients with | | | |
|--|----------------------|--------------------|---|-------------------|
| | oral lichen planus | | recurrent aphthous ulcerations (n = 13) | controls (n = 50) |
| | erosive (n = 32) | reticular (n = 25) | | |
| Age (median, range; y) | 58 (30-72) | 35 (20-52) | 62 (40-74) | 52 (19-70) |
| Sex (m/f)* | 7/25 | 5/20 | 5/8 | 15/35 |
| Smokers/non-smokers | 6/28 | 4/21 | – | 6/44 |
| Autoantibodies (U/mL, median, interquartile range) | | | | |
| anti-Dsg 1 | 10.4 (4.9-16.3)† | 2.3 (1.5-4.9) | 3.0 (1.4-4.3) | 2.9 (2.0-4.7) |
| anti-Dsg 3 | 9.1 (3.3-12.9)† | 2.4 (1.1-3.1) | 1.5 (0.5-2.3) | 2.9 (2.0-5.0) |
| IIF (positive/tested) | 18/22‡ | 3/15 | 1/10 | 1/20 |

*Abbreviations: m – male; f – female; Dsg – desmoglein; IIF – indirect immunofluorescence.

† $P < 0.001$ vs other groups (Kruskal-Wallis test with Mann-Whitney post-hoc analysis).

‡ $P < 0.001$ vs other groups (χ^2 test).

were within the ranges found in healthy controls (Table 1). Concentrations of both desmoglein autoantibodies were significantly higher in patients with erosive form of oral lichen planus than in healthy controls ($P < 0.001$ for both autoantibodies), patients with recurrent aphthous ulceration ($P < 0.001$ for both autoantibodies), and patients with reticular oral lichen planus ($P < 0.001$ for both autoantibodies). Indirect immunofluorescence analysis using rat esophagus and/or rat bladder as substrates showed significantly more findings positive to epithelial structures in patients with erosive oral lichen planus (18 positive of 22 tested) than in other three groups (Table 1). There was no difference in indirect immunofluorescence findings between the patients with recurrent aphthous ulcerations, patients with reticular form of oral lichen planus, and healthy controls (Table 1).

Discussion

Increased concentrations of both anti-desmoglein 1 and anti-desmoglein 3 autoantibodies, which were detected in the sera of patients with erosive form of oral lichen planus and confirmed by indirect immunofluorescence, indicated that anti-keratinocyte autoantibodies may be involved in pathogenesis of this clinical form of oral lichen planus. To the best of our knowledge, this is the first report on anti-desmoglein 1 and anti-desmoglein 3 circulating autoantibodies in patients with idiopathic oral lichen planus.

Ingafou et al (12) reported that oral lichen planus was not associated with IgG circulating antibodies to epithelial antigens. However, they used indirect immunofluorescence to determine autoantibodies to the epithelial components of monkey esophagus rather than desmoglein autoantibodies. We also performed indirect immunofluorescence using the same substrate and found 21 positive among 37 tested patients with oral lichen planus, ie, 2.5-fold more positive findings in comparison with results reported by Ingafou et al (12). This difference might be attributed to different serum dilutions used for indirect immunofluorescence (1:100 dilution in Ingafou's study vs 1:20 used in ours). However, these authors found significantly more positive findings in patients with erosive than in those with reticular form of oral lichen planus, which is in accordance with our results. Increased concentrations of both desmoglein autoantibodies in patients with erosive form of oral lichen planus and significantly higher proportion of indirect immunofluorescence-positive findings in patients with erosive than in patients with reticular form of oral lichen planus might indicate significant differences between these two clinical manifestation of oral lichen planus regarding their possible immunopathological features. In a previous study, we speculated that a new form of the disease may be manifested in the form of erosive oral lichen planus, or that overlapping of two diseases – erosive lichen and an autoimmune bullous disease – might have occurred, as already reported by some

authors (13,21,22). Presently, it is not clear if anti-desmoglein antibodies in erosive oral lichen planus could be of primary pathogenic significance or might have appeared as the result of epitope spreading process, known to be present in autoimmune diseases (23). During the process of antigen-specific (24,25) or non-specific (26) keratinocyte damage, antigenic material, including desmogleins, may be released and may elicit autoantibody generation. This process does not seem to be equally represented in erosive and reticular forms of oral lichen planus, as indicated by more prominent expression of anti-desmoglein autoantibodies in erosive form.

Patients with recurrent aphthous ulceration served as another control group because oral lichen planus and recurrent aphthous ulceration, although different in clinical manifestation and natural history, seemed to share immunopathological features involving T cell-mediated immunity (17,27). Our results indicate that these two diseases do not share similar features with respect to humoral immunity. Concentrations of both anti-desmoglein 1 and anti-desmoglein 3 antibodies in patients with recurrent aphthous ulceration were in the range of those found in healthy controls. Only 1 of 10 patients with recurrent aphthous ulceration was indirect immunofluorescence-positive when monkey esophagus was used as a substrate, whereas all were negative when rat bladder was used (data not shown).

In conclusion, humoral immunity against keratinocyte cadherins desmoglein 1 and desmoglein 3 seems to play a role in oral lichen planus. Significant differences in their serum concentrations may be either indicative of different immunopathogenic mechanisms involved in erosive and reticular oral lichen planus, or just reflect the differences in pathophysiology of these two forms of oral lichen planus.

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