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AN IMMUNOFLUORESCENCE STUDY ON THE HUMORAL IMMUNITY TO ADULT HUMAN ORAL MUCOSA IN RECURRENT APHTHOUS STOMATITIS

By

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Microbiological and immunological investigations on the aetiology of recurrent aphthous stomatitis (syn. recurrent aphthous ulceration) have dealt with the role of virus (4, 21, 22, 23, 24, 26), the possibility of streptococcal hypersensitivity (1, 10, 12, 13, 14, 15), and of autoimmune disease (5, 7, 8, 9, 16, 17, 18, 19, 20).

In support of an autoimmune mechanism, haemagglutination, complement fixation, and precipitation tests have shown elevated antibody-titres to an antigenic extract of foetal human oral mucosa in patients suffering from recurrent aphthous ulceration (17). In a lymphocyte transformation test, lymphocytes from patients with recurrent aphthous ulceration were stimulated by the same foetal human oral antigen extract (18).

Further studies have demonstrated cytotoxic effects by lymphocytes from patients with recurrent aphthous ulceration upon adult human oral epithelial cells from gingival tissue (7). Inhibition of the lymphocyte cytotoxicity was achieved by preincubation of the lymphocytes in rabbit anti-human lymphocyte serum (9), or by addition of hydrocortisone to the cell cultures (8).

The results of immunofluorescence studies on the humoral

antibodies to adult human oral mucosa are uncertain (5, 19). The material available is limited, but Lehner's study (19) indicates that sera from patients with recurrent aphthous ulceration contain IgG and IgM antibodies to the cytoplasm of autologous stratum spinosum cells of the oral epithelium. Sera from controls did not contain any demonstrable antibodies to autologous oral mucosa.

The purpose of the present investigation was to study, with the help of the immunofluorescence (IF) technique, the humoral immunity to *adult* human oral mucosa in patients with recurrent aphthous stomatitis of the simple type (RAS).

MATERIALS AND METHODS

Recurrent aphthous ulceration (syn. recurrent aphthous stomatitis) is classified into simple, cicatricel, and herpetiform types (11). In the present study, only patients with the simple type of recurrent aphthous stomatitis (RAS) were investigated. The material consisted of sera from 24 patients with RAS and sera from 24 persons without any anamnestic or clinical evidence of RAS.

Humoral antibodies were detected by a double-layer immunofluorescence staining method. The antigens used were unkeratinized buccal human oral mucosa from a patient with RAS; unkeratinized buccal human oral mucosa from a control person; adult human skin; adult human smooth muscle; and adult guinea-pig oral mucosa from the lower lip. Both the patient and the control person were blood-group O Rh-negative. The biopsies from human oral mucosa and skin, and the biopsies from guinea-pig oral mucosa were quick-frozen in cooled pure isopentane, and stored at -20°C . The human smooth muscle tissue was quick-frozen in dry-ice, and stored at -80°C . All the tissues were cut on a cryostat at -30°C , mounted on slides, and air-dried for 15 min at room temperature before use.

The titration of antisera, the conjugates, the IF staining, and the microscopic system have previously been described (6, 13). The magnification used in the present study was $250\times$, using a $25\times$ plane fluorite objective NA 0.5 and $8\times$ periplane eyepieces. The IF staining of the human oral mucosal tissues and the adult human smooth muscle was done twice with all 48 sera in all titrations used. The results were read as positive or negative. Positive reactions appeared as a green fluorescence in the cytoplasm of the stratum spinosum cells in the human oral epithelium (Fig. 1). On every day of examination, titrated series from 4 probands (2 patients and 2 controls) were read blindly. To ensure that a positive fluorescence staining of the oral mucosa was specific, the experiments summarized in Table 1 were carried out. The absorption of the 48 sera with adult human oral mucosa was performed in 2-3 h at 21°C using homogenized



Fig. 1.

Stratum spinosum cells of oral epithelium showing positive immunofluorescence after staining with a double layer immunofluorescence staining method. $\times 348$.

tissue mixed in equal parts with diluted, but strongly positive, sera. Adult human skin and adult guinea-pig oral mucosa were further stained with 11 of the 24 sera from patients with RAS which showed positive reactions to adult human oral epithelium. These stainings, too, were performed twice in all titrations used.

RESULTS

The distribution of the antibody endpoint titres against the cytoplasm of the stratum spinosum cells of adult human oral mucosa in 24 controls and 24 patients with RAS is shown in Figs. 2, 3 & 4. No differences in endpoint titre were demonstrated using human oral mucosa from the patient with RAS or from the control person.

The histograms and the cumulative distributions indicate that the controls and the patients represent two different populations. The antibody titres of the controls and the pa-

TABLE 1

Reactions for Establishing Specificity of a Positive Human Oral Mucosa Immunofluorescence Staining.

Antigen	Antisera	Conjugate	Results
Human oral mucosa	Phosphate buffered saline	RaAH Ig/FITC	Negative
Human oral mucosa	Positive serum absorbed with human oral mucosa	RaAH Ig/FITC	Negative
Human smooth muscle	Positive serum	RaAH Ig/FITC	Negative
Human oral mucosa	Positive serum	RaAH Ig/FITC	Positive

NUMBER OF CASES

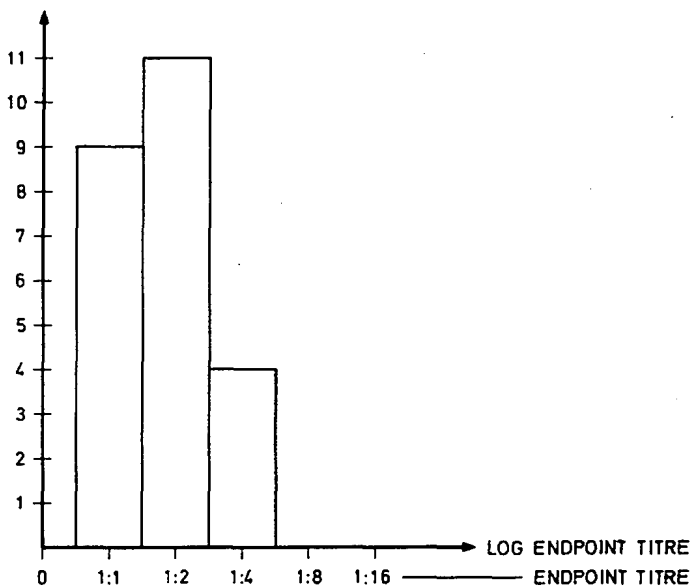


Fig. 2.

Histogram showing the distribution of humoral antibodies to the stratum spinosum cells of human oral mucosa in 24 controls. The endpoint titres were obtained by immunofluorescence staining.

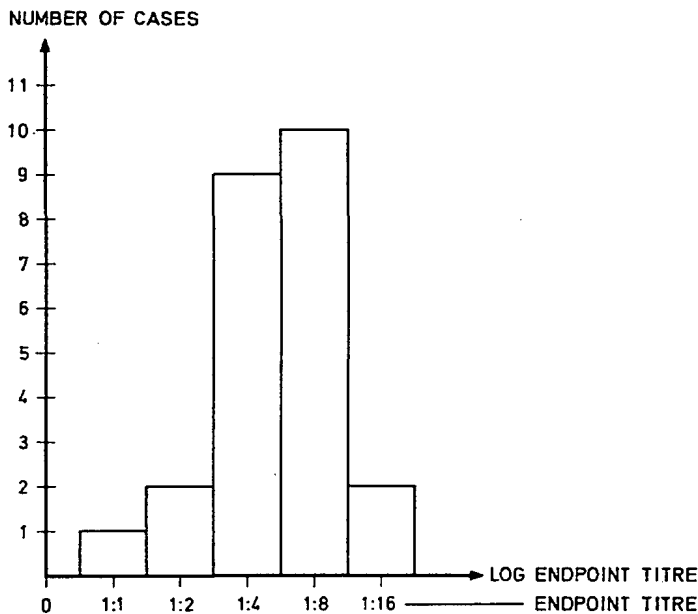


Fig. 3.

Histogram showing the distribution of humoral antibodies to the stratum spinosum cells of human oral mucosa in 24 patients with RAS. The endpoint titres were obtained by immunofluorescence staining.

tients ranged from 1:1 to 1:4 and from 1:1 to 1:16 respectively.

Statistical analysis using the Mann-Whitney U-test (25) shows that the mean value of the endpoint titre in the group of controls is significantly smaller than the mean value of the endpoint titre in the group of patients with RAS ($P < 0.001$).

The sera from 15 patients who were in an active phase of the disease showed endpoint titres ranging from 1:1 to 1:16. The endpoint titres in the sera from 9 patients who were in an inactive phase ranged from 1:4 to 1:8. The two groups showed no significant difference in the distribution of end-

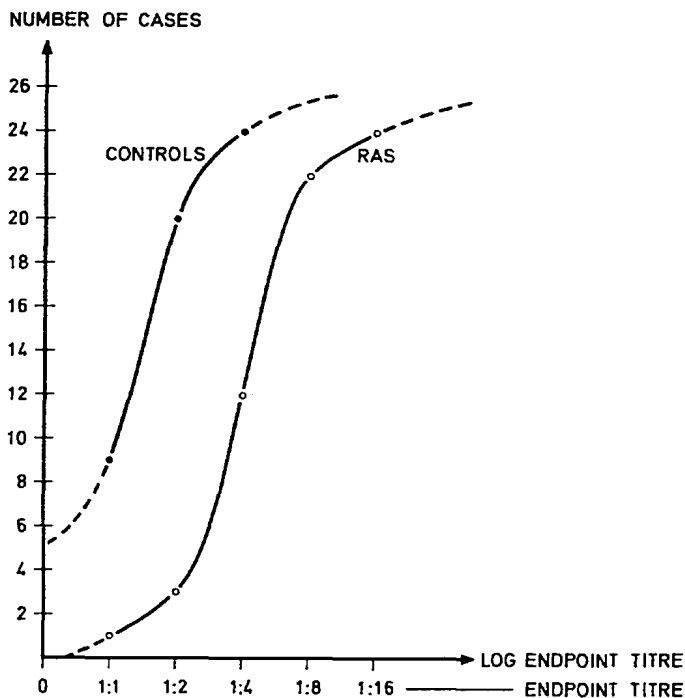


Fig. 4.

Cumulative distributions of the histograms in Fig. 2 and Fig. 3. (Sign. - - - - controls, - O - O - O - RAS).

point titres ($P > 0.10$). No significant difference in the distribution of endpoint titres was demonstrated in relation to sex ($P > 0.10$).

The control reactions, with phosphate buffered saline as the first layer, were all negative. The absorption with oral mucosa succeeded in all 48 sera since the slides in the following set-up series were all negative. All the sera from the controls and from the patients with RAS showed negative reactions to human smooth muscle tissue. Antibodies to adult human skin were not detected in any of the 11 sera from patients with

RAS. All the 11 sera showed antibodies reacting with adult guinea-pig oral epithelium. The endpoint titres to adult guinea-pig oral mucosa were always lower than, or equal to, the endpoint titres to adult human oral mucosa.

DISCUSSION

The present study shows that it was possible, with the help of IF staining and the fluorescence microscope system, to demonstrate antibodies to adult human oral mucosa obtained from a patient with RAS as well as to adult human oral mucosa obtained from a control person. These positive reactions were demonstrated both in the patients with RAS and in the controls. The results show a quantitative difference of the antibody titre to oral mucosa in the patients with RAS in relation to the controls, and thus support the autoimmune theory (5, 7, 8, 9, 16, 17, 18, 19, 20). The possibility that non-specific oral mucosal damage may induce the formation of antibodies to the oral mucosa was not investigated in the present study. However, Lehner (16) did not demonstrate any significant difference in the antibody titre to foetal human oral mucosa in patients with other ulcerating oral diseases in relation to controls without any oral disease.

The variation in antibody titre in the patients in the present study is similar to the variation of antibody titre to foetal human oral mucosa (16). The antibody titres are lower in the present investigation. This lower concentration of antibodies could be due to differences in the antigen used and/or a variation in the degree of sensitivity to oral mucosa in the two groups of patients investigated. The sera with the highest antibody titres to adult human oral mucosa were found in patients with recurrences. As each patient was tested only once, the present IF study does not show if the titre of antibodies to adult human oral mucosa is related to the recurrence of disease.

The finding of antibodies to human oral mucosa in the con-

trols, and the demonstration of positive IF when using sera from patients on normal homologous oral mucosa, are at a variance to Lehner's findings (19). The discrepancy may be due to the possibility that the IF technique used in the present study is a more sensitive technique for detecting humoral antibodies.

The conjugate used in the present investigation was a mixture of antihuman IgG and IgM. Therefore, our results support Lehner's study (20) which shows that the auto-antibodies in RAS belong to the immunoglobulin classes IgG and IgM. As no attempt was made to detect IgA against adult human oral mucosa, the present IF study does not denote the binding of IgA and the decreased serum concentration of IgA in RAS reported by Brody & Silverman (5). Like Lehner (19), we only demonstrated specific immunofluorescence in the cytoplasm of the epithelial cells and did not demonstrate specific immunofluorescence in the connective tissue.

The specificity of the IF staining was controlled by the negative results when using phosphate buffered saline or absorbed sera as the first layers. As the immunofluorescence was localized to the cytoplasm of the oral epithelial cells, and as the human oral mucosal tissues used were O Rh-negative, the positive reactions demonstrated are not due to ABO or rhesus blood-group incompatibility. The antibody nature of the serum factors responsible for the staining reactions of the cytoplasm in epithelial cells has been discussed and is still obscure (2, 3). However, the demonstration of a significantly quantitative difference in endpoint titre in patients with RAS in relation to controls, and the negative result obtained when adult human skin was used as antigen seem to indicate that some of the immunofluorescence of the cytoplasm may be due to a specific antigen-antibody reaction. The negative staining reactions in the present study for adult human muscle tissue and skin, and the positive reactions for adult guinea-pig oral mucosa suggest the possibility that the antibodies to adult human oral epithelium might be organ-specific, but not species-specific. Any final conclusions on the cross-reactions are not possible for the pre-

sent, but need further investigations using other adult tissues, especially other epithelial tissues.

One might claim that the adult tissues used could be microbiologically contaminated. On the other hand, in Donatsky's study (10) bacteria were demonstrated only in the aphthous ulcerations, and never in the normal epithelial cells in the unaffected oral mucosa in relation to the aphthous ulcers. Further, it seems reasonable to suggest that a diffuse intercytoplasmatic accumulation of virus would reveal alterations demonstrable not only by electron microscopy, but also by light microscopy. No alterations of the epithelial cells were seen by examination in the light microscope.

The present results indicate that a difference in humoral immunological response to normal adult human oral mucosa could be involved in RAS. The results are in agreement with a similar difference in the cellular immunological response demonstrated in the target cell destruction test (7). The occurrence of antibodies to adult human oral mucosa in other oral diseases was not investigated. Therefore, we conclude that the present IF study shows that antibodies to adult human oral mucosa are a feature of recurrent aphthous stomatitis.

SUMMARY

The occurrence of humoral antibodies to *adult* human oral epithelium in recurrent aphthous stomatitis (RAS) was investigated by titrating sera, and by using a double-layer immunofluorescence staining method. The material consisted of 24 patients with RAS and 24 persons without anamnestic or clinical signs of RAS. The mean value of the endpoint titre in the group of controls was significantly smaller than the mean value of endpoint titre in the group of patients with RAS ($P < 0.001$). No antibodies to human smooth muscle or skin were demonstrated. Sera with known activity against adult human oral epithelium showed positive reactions to adult guinea-pig oral epithelium. The results show that an increase in antibody titres to adult human oral mucosa is a feature of RAS.

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