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IN VITRO DEMONSTRATION OF CELLULAR
HYPERSENSITIVITY TO STREP 2A
IN RECURRENT APHTHOUS STOMATITIS
BY MEANS OF THE
LEUCOCYTE MIGRATION TEST

By

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Recurrent aphthous stomatitis (RAS) (syn.: recurrent aphthous ulceration, recurrent aphthous ulcers, recurrent oral ulceration, recurrent oral aphthae, and recurrent Mikulicz's aphthae) is a disease of the oral mucosa characterized by recurrent, burning, smarting, painful, single or multiple, erosive or fibrinopurulent, well demarcated ulcerations with a peripheral red halo (8).

Although the aetiology of RAS is still unknown, studies by *Lehner* (12, 13, 14, 15, 16, 17, 18, 19), *Brody & Silverman* (5), and *Dolby* (6, 7) suggest an autoimmune mechanism. Studies by *Barile et al.* (1, 3), *Barile & Graykowski* (2), *Graykowski et al.* (10, 11), and *Stanley et al.* (20) indicate another aetiology associated with microbial infection. From multiple isolations in recurrent aphthous lesions they obtained pure cultures of *Strep sanguis* prototype 2A, N.I.D.R. By microscopic examination microorganisms morphologically consistent with the pleomorphic *Strep* 2A could be demonstrated in the majority of microscopic sections from aphthous lesions. A possible pathogenetic role of these microorganisms further

appeared from the fact that intracutaneous injection of Strep 2A was able to induce a delayed type hypersensitivity reaction in patients with RAS. As a result of these examinations it was therefore suggested that RAS may represent a specific streptococcal hypersensitivity, and that the intracellular, inactive L-form of Strep sanguis isolated from some aphthous ulcerations may be responsible for the recurrence of attacks. This theory, however, was not confirmed by *Francis & Oppenheim* (9), who found impaired lymphocyte stimulation by Strep 2A in RAS, indicating immunological hyporesponsiveness, i.e. deficiency of the cellular response to Strep 2A.

The purpose of the present study was on this background by means of the leucocyte migration test (LMT) (4, 24) to investigate the occurrence of cellular hypersensitivity against Strep 2A in patients with RAS.

MATERIAL AND METHODS

Seventeen patients (12 females, 5 males, age 20-68 years, average 40 years) with clinically characteristic RAS, and 13 persons (6 females, 7 males, age: 20-70 years, average 46 years) without any clinical or anamnestic evidence of the disease were examined.

The leucocyte migration technique (LMT) was performed according to the description by *Søborg & Bendixen* (24) and *Bendixen & Søborg* (4). Leucocytes from the peripheral blood are washed four times and transferred to capillary tubes, which are placed at the bottom of tissue culture chambers containing 1 ml of tissue culture medium (TC 199) with 10 per cent horse serum. The cell migration from the open end of the capillary tube is measured by planimetry after 24 hours. The migration areas of a series of antigen-containing cultures (Ma) and the migration areas of a series of control cultures (Mo) are used for calculation of the migration index (MI) in the following way:

$$MI = \frac{Ma}{Mo}$$

MI < 1.0 therefore indicates inhibition, MI > 1.0 stimulation of the migration.

Preparation of antigen. Strep sanguis prototype 2A (kindly provided by dr. E. A. Graykowski, San Francisco, California) was used as antigen. The bacteria were subcultured on horse blood agar and aerobically multiplied on membranes of Cellophane® on horse blood agar by incubation at 37°C for 24-48 hours. Before washing with 0.9 per cent NaCl microscopic smears were examined from each membrane to control the purity of the cultures. The streptococci were

concentrated by centrifugation ($3400 \times G$ for 20 minutes). By direct counting technique the streptococcal suspension was adjusted at 500×10^6 organisms per ml ± 10 per cent and heat killed at $60^\circ C$ for 30 minutes. In preliminary experiments the highest, non-toxic concentration of streptococci in the LMT was determined to be 5×10^6 organisms per ml culture medium, and this amount was used as routine in the LMT.

RESULTS

Table 1 shows the migration indices of 13 control persons and 17 patients with RAS. From *Figure 1* it can be seen that the patients seem to fall in three categories, 8 patients showing inhibition of the migration, 1 patient stimulation, and 8 patients neither inhibition nor stimulation. When the patients

TABLE 1

MI Controls (13)	MI RAS (17)
1.07	0.77
0.97	0.74
0.96	0.85
1.08	0.94
1.04	0.76
1.01	1.01
0.95	1.09
0.88	0.75
1.00	0.86
0.89	0.84
0.93	1.02
1.01	0.78
1.00	0.84
	1.00
	0.94
	1.14
	1.05
mean 0.98	mean 0.90
SD 0.06	SD 0.13
95 % range 0.86-1.10	95 % range 0.64-1.16

Leucocyte migration test with Strep 2A (5×10^6 organisms per ml). Migration indices (MI) of 13 control persons and 17 patients with recurrent aphthous stomatitis (RAS).

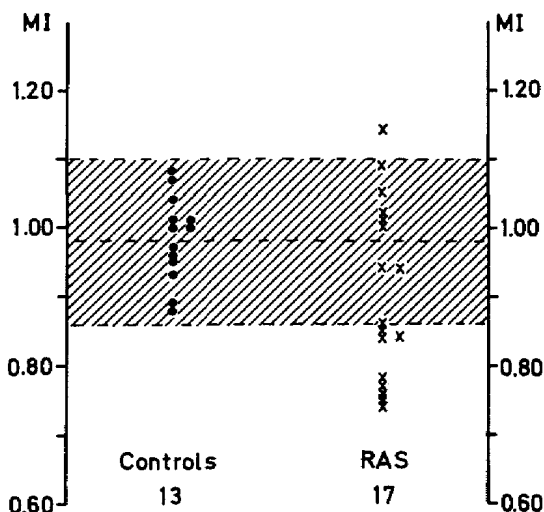


Fig. 1.

Leucocyte migration test with Strep 2A (5×10^6 organisms per ml) showing the migration indices (MI) of 13 control persons and 17 patients with recurrent aphthous stomatitis (RAS). Hatched area: 95 per cent range of normal control persons. Graphic presentation of figures in Table 1.

with RAS are regarded as one group, the mean MI is 0.90 with a standard deviation of 0.13. The mean MI of the control is 0.98 with a standard deviation of 0.06. Calculation by F-test shows that the difference in standard deviation between the two groups is statistically significant ($P < 0.01$) and indicates that the patients and the controls form two different populations. Calculation using the method of Welch further shows that the difference between the mean MI values of the two groups is significant ($0.01 < P < 0.025$).

DISCUSSION

Antigen-induced inhibition of the in vitro migration of peripheral human leucocytes is a well established parameter of cellular hypersensitivity (21). The present results indicate

that cellular hypersensitivity against Strep 2A was present in 8 of 17 patients with RAS, but was not demonstrated in 13 consecutive, normal control persons. This finding confirms an association between RAS and Strep 2A, which has previously been demonstrated by intracutaneous reactions (10), and is at a variance with the immunological hyporeactivity to Strep 2A, as demonstrated in lymphocyte transformation experiments (9). This discrepancy, however, may be a reflection of the fact, that antigen-induced lymphocyte transformation is qualitatively uncorrelated to antigen-induced migration inhibition as shown by Søbørg *et al.* (23).

Strep 2A did not induce migration inhibition in all cases of RAS. One patient showed antigen-induced stimulation of the migration, and 8 patients had values of MI within the normal range. The findings in the latter cases, however, do not immediately exclude the presence of cellular hypersensitivity. According to Søbørg's analysis of the LMT in brucella hypersensitivity (22), a fixed, relatively low concentration of antigen induces migration inhibition in cultures of high sensitivity, and migration stimulation in cultures of low sensitivity, whereas cultures with intermediary sensitivity show neither inhibition nor stimulation. As in previous studies with the LMT (25, 26) the antigen used in the present investigation possessed a non-specific toxicity, which did not permit the use of high concentrations in the migration cultures, and systematic titrations of the LMT reactions, therefore, could not be performed.

The significantly higher standard deviation of MI values in the patient group than in the controls—including one case of antigen-induced stimulation—may therefore express intermediary, somewhat varying degrees of cellular hypersensitivity in some patients with MI values within the normal range. Conclusive information on this point presupposes LMT-titration with a less toxic, antigenic component from Strep 2A, and has not yet been performed. The true incidence of cellular hypersensitivity to Strep 2A in RAS, therefore, cannot be evaluated on basis of the present study. But the

investigation has confirmed, by examinations in an in vitro system, that Strep 2A hypersensitivity of the cellular type is a feature of RAS. The LMT may thus be a useful tool in future explorations of the role of streptococcal hypersensitivity in the pathogenesis of RAS.

SUMMARY

An in vitro demonstration of cellular hypersensitivity to Strep 2A in 9 of 17 patients with recurrent aphthous stomatitis (RAS) was accomplished by means of the leucocyte migration test. Reactivity to the same antigen dose was not present in 13 consecutive normal controls. For methodological reasons, which are discussed, the number of positive reactions found represent a minimum, and it cannot be excluded therefore, that the true incidence of cellular hypersensitivity against Strep 2A in RAS is considerably higher than shown.

ACKNOWLEDGMENT

The study was supported by "The Danish Hospital Foundation for Medical Research. Region of Copenhagen, Faroe Islands and Greenland".

The authors are grateful to Dr. K. Stolze for statistical advice.

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