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DELAYED HYPERSENSITIVITY TO CANDIDA
ALBICANS IN MAN
DEMONSTRATED IN VITRO: THE CAPILLARY
TUBE MIGRATION TEST

By

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In 1963 *George & Vaughan* produced evidence that specific inhibition of migration of macrophages by antigens was an in vitro correlate of delayed hypersensitivity of animals. Since then their capillary tube technique has been modified and used for the in vitro study of human delayed hypersensitivity. Using cells obtained from human lymph nodes *Thor* (26) was able to demonstrate delayed hypersensitivity to microbial antigens. *Søborg & Bendixen* (19) demonstrated specific inhibition of migration of *peripheral* leucocytes isolated from patients showing a delayed hypersensitivity skin reaction to brucella. *Søborg & Bendixen's* modification of the capillary tube technique has been successfully used in studies on human tuberculin hypersensitivity by *Clausen & Søborg* (7) and *Nordquist & Rorsman* (15), in studies on allergy to neomycin by *Nordquist & Rorsman* (14), and in studies of delayed hypersensitivity in ulcerative colitis, glomerulonephritis, Hashimoto's disease and Sjögren's syndrome by *Bendixen* (2, 3), *Søborg & Bertram* (24), and *Søborg & Halberg* (23).

Delayed hypersensitive reactivity to *C. albicans* is widely distributed in the adult population. *Shannon et al.* (18) and *Holti* (12) found a positive skin test to *C. albicans* in 50-85

per cent of non-infected persons. A positive skin test to *C. albicans* therefore is of no value in establishing evidence of a candida infection as found by *Comaish et al.* (8), whereas a high antibody titer is more informative as reported by *Comaish et al.* (8), *Lehner* (13), and *Budtz-Jørgensen* (4). However, the assessment of the integrity of delayed hypersensitivity reactions may be useful, as impairment of cellular immunity was found by *Canales et al.* (5), *Chilgren et al.* (6), *Folb & Trounce* (10), and *Valdimarsson et al.* (27) to be of critical importance in the development of systemic or muco-cutaneous candidiasis.

The present investigation was undertaken to study the applicability of Søborg & Bendixen's modification of the capillary tube technique for in vitro demonstration of delayed hypersensitivity to *C. albicans*. Both cell suspensions and an extract of disintegrated candida cells were used.

MATERIAL AND METHODS

The material comprised 45 healthy persons under no medical treatment which might influence the state of cellular hypersensitivity.

Tissue Culture Technique

The leucocyte migration test was carried out employing the method described by Søborg & Bendixen (19). The leucocytes were obtained after spontaneous sedimentation of 30 ml of heparinized venous blood at 37° C for 1 hour. From each 30 ml blood 10–12 capillary tubes were drawn. The cells were washed 3 times in Hank's balanced salt solution. At the fourth and final washing in medium (TC 199, Difco) the samples were mixed in order to ensure a homogenous composition. TC 199, Difco, was used as culture medium with an admixture of 10 per cent horse serum, penicillin, and streptomycin. The pH was adjusted to 7.3.

In each person a leucocyte migration assay was made two times with an interval of 2–14 days.

Preparation of Antigen

Cell-suspensions of *C. albicans* type A, standardized to a strength of 5×10^7 , 1×10^8 , and 2×10^8 by a haemocytometer count, were prepared in a 0.9 per cent NaCl—phosphate buffer (pH 7.2). The cells were washed 3 times and killed by heating for 1 hour at 60° C.

A saline extract of ultracentrifuged, disintegrated cells of *C. albicans* type A

was prepared according to *Axelsen* (1). The candida extract was kept frozen at $\div 25^{\circ}$ C until immediately before use.

Leucocyte Migration Assay

Of the 10-12 capillary tubes 3 were used for control cultures. To the remaining 7-9 cultures 100 μ l of antigen (cell-suspension or extract) in varying concentrations was added with 2-3 identically treated cultures for each strength of antigen. The final concentration of Candida cells in the culture chamber was 5 mill., 10 mill. or 20 mill. per ml. The chambers were sealed and incubated for 24 hours at 37° C. After incubation the migration areas were studied in a projection microscope and the area was traced on paper of constant weight according to *Ferraresi et al.* (9). The tracings were cut out and weighed on a Mettler analytical balance. The migration index (MI) was calculated for each antigen suspension by comparing the mean of the migration in the presence of antigen (MX) to that in the absence of antigen (MO).

$$MI = \frac{MX}{MO}$$

As all leucocyte migration assays were conducted twice the migration index was calculated on 4-6 identically treated samples.

Skin-testing

After the leucocyte migration test was carried out an in vivo assay of delayed hypersensitivity to *C. albicans* was made by intracutaneous injection of 0.1 ml of the aforementioned extract of *C. albicans* in a 1/1000 dilution. The skin reaction was read after 48 hours. The reaction was recorded as positive when the induration was 5 mm or more.

RESULTS

Fig. 1 shows the distribution of the migration indices of 45 consecutive persons (17 persons with a negative intra-cutaneous reaction and 28 persons with a positive intracutaneous reaction). The concentration of candida cells in the culture chambers was 10 mill. per ml. The average migration index for the candida-negative persons was 0.89 ± 0.06 (standard deviation) and that for the candida-positive persons 0.69 ± 0.09 . Analyzed with the Students *t* test these two populations were significantly different ($P < 0.001$).

The lower limit for the normal range is $0.89 \div 2 \times 0.06 = 0.77$. From Fig. 1 it can be seen that there is some

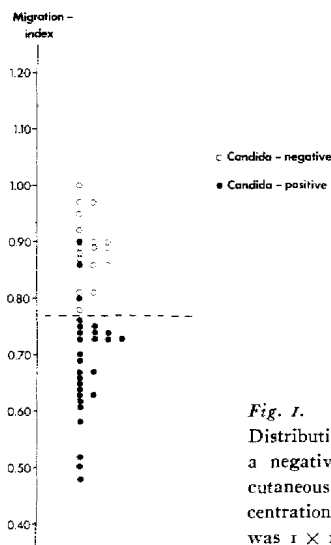


Fig. 1.

Distribution of migration indices of 17 persons with a negative and 28 persons with a positive intracutaneous reaction to a candida extract. The concentration of candida cells in the tissue chambers was 1×10^7 per ml.

overlapping between the groups as the migration index for three of the candida-positive is in the lower half of the normal range.

A final concentration of 10^7 candida cells per ml in the culture chambers was found to be the optimum for in vitro detection of delayed hypersensitivity to *C. albicans*. When using 5 mill. candida cells per ml inhibition of migration of the candida-positive was less significant. On the other hand, pronounced non-specific inhibition of migration of the candida-negative was seen when the concentration of candida cells was 20 mill.

Fig. 2 shows the relationship between the size of the indurated area 48 hours after intracutaneous injection of a saline extract of *C. albicans* and the migration index in both candida-positive and candida-negative individuals. A strong negative correlation was observed of $r = -0.75$, $P < 0.001$. However, this correlation was not significant for the group

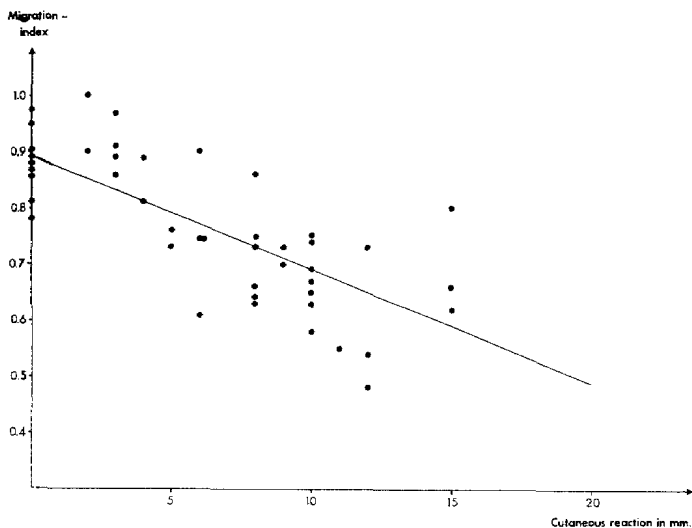


Fig. 2.

The relationship between the intracutaneous reaction and the inhibition of migration.

of candida-positive when excluding those with a negative intracutaneous reaction ($r = \div 0.35$, $0.05 < P < 0.1$).

Fig. 3 shows the distribution of migration indices of 40 consecutive patients (17 with a negative and 23 with a positive intracutaneous reaction) when using a saline extract of *C. albicans* as antigen in the culture chambers. The mean value of the migration indices for the candida-negative was 0.97 ± 0.08 , while the mean value for the candida-positive was 0.90 ± 0.19 . The difference in the mean value between the two groups was not significant ($t = 1.44$, $0.1 < P < 0.2$). However, the difference in standard deviation indicates that candida-positive and candida-negative form two significantly different populations ($F = 6.0$, $P < 0.01$).

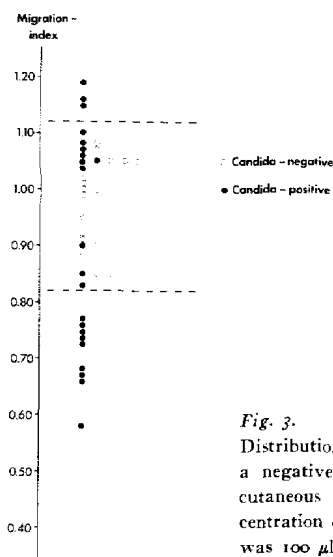


Fig. 3.

Distribution of migration indices of 17 persons with a negative and 23 persons with a positive intracutaneous reaction to a candida extract. The concentration of candida extract in the tissue chambers was 100 μ l per ml.

DISCUSSION

The present study has shown that specific inhibition of migration of peripheral blood leucocytes occurs in persons showing a positive intracutaneous reaction to a candida extract when cell-suspensions of *C. albicans* are used in the culture chambers. The optimum concentration of antigen was found to be 10 mill. cells per ml and at this antigen-concentration a migration index of ≤ 0.77 was a clear indication of delayed hypersensitivity. However, indices of > 0.77 may be found in sensitive persons.

When using a saline extract of *C. albicans* as antigen both inhibition and stimulation of migration as well as apparent unreactivity were seen among persons showing a positive intracutaneous reaction. According to Søbørg (21, 22) inhibition of migration indicates a high degree of sensitivity, whereas stimulation of migration reflects a less sensitive state.

Migration indices falling within the normal range may then represent either an intermediary degree of sensitivity or no sensitivity at all. In the present study a diluted, however, according to *Axelsen* (1), highly potent antigen was used. As *Søborg* (21, 22) and *Rosenberg & David* (17) have shown that high concentrations of antigen result in inhibition and low concentrations in stimulation of migration a more distinct separation between sensitive and non-sensitive persons would probably have occurred in the present study if higher antigen concentrations had been used.

In *Søborg's* study (20) with brucella as antigen inhibition of migration was seen to some extent even among brucella-negative persons (0.92 ± 0.07). The explanation given for this non-specific inhibition of migration was that the high antigen concentrations used in the experiments (50 mill. micro-organisms per ml) were slightly toxic for normal cells. In the present study a slight inhibition of non-sensitive leucocytes occurred at an antigen concentration of 10 mill. micro-organisms per ml (0.89 ± 0.06). When higher antigen concentrations were used (25 mill. micro-organisms per ml) the non-specific inhibition of migration was more pronounced. It therefore appears as though *C. albicans* is more toxic to leucocytes than brucella, a fact which may obscure the specific inhibition of migration. It is possible, however, that the increased non-specific inhibition of migration with candida-cells as antigen is partly due to a delayed migration of the leucocytes into the culture chamber and not entirely an expression of a toxic damage of the leucocytes. The large diameter of candida cells as compared with for instance brucella, may produce a mechanical barrier for the leucocytes which detains their migration.

Studies by *O'Neill & Favour* (16), *Thor & Dray* (26), and *Rosenberg & David* (17) have failed to show any correlation between the inhibition of migration and the size of the intracutaneous reaction when human cell-cultures were used. *Søborg* (20) however, was able to demonstrate a clear correlation between the two parameters of delayed hyper-

sensitivity in brucella-positive persons. As in *Clausen & Søborg's* study on tuberculin hypersensitivity (7) the present study showed good correlation between the size of the intracutaneous reaction and the inhibition of migration when persons with a negative intracutaneous reaction were included in the analyzed material. This correlation was not significant for the group of persons with an intracutaneous reaction of ≥ 5 mm. However, if the investigation had comprised a larger number of persons with the same distribution as the present the correlation would have reached the level of significance. As it is very difficult to standardize the experimental conditions when using the intracutaneous skin test the leucocyte migration test may well be a better quantitative estimate of delayed hypersensitivity in man.

The present study has provided further evidence that the capillary tube migration technique is a reliable test for in vitro detection of delayed hypersensitivity to microbial antigens. In future it may be a valuable test for the demonstration of changes in the level of cellular immunity in candidiasis.

SUMMARY

In the present study Søborg and Bendixen's modification of the capillary tube migration technique has been successfully used as an in vitro test for detection of delayed hypersensitivity to *C. albicans*. When suspensions of *C. albicans* were used in the culture chambers specific inhibition of migration of peripheral leucocytes was seen in persons with a delayed hypersensitive skin reaction after intracutaneous injection of a candida extract. When a saline extract of disintegrated candida cells was used as antigen in the culture chambers both inhibition and stimulation of migration occurred which indicates that the antigen concentration was too diluted. Among the skin-test positive there was a poor correlation between the inhibition of migration and the size of the indurated area after intracutaneous skin-testing.

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