

THE LEUKOCYTE MIGRATION TEST IN ATOPIC DERMATITIS

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Abstract. Leukocyte migration tests with a human dander extract were performed in 16 patients with atopic dermatitis and in 10 normal controls. Significant inhibition as well as stimulation of migration of leukocytes were observed in 11 of the 16 patients with atopic dermatitis. Inhibition of migration could be elicited only in those cases where delayed skin reactions to the dander were strongly positive. These results indicate that a state of delayed hypersensitivity against components of human dander exists in some patients with atopic dermatitis.

Many investigators have reported that delayed intracutaneous reactions to fungal, bacterial, viral and inorganic substances are not prevalent in patients with atopic dermatitis (7, 8, 9, 10, 11, 16). However, we have previously shown that the frequency of positive delayed intracutaneous reactions to a human dander extract is significantly higher in patients with atopic dermatitis than in non-atopic controls (16).

The leukocyte migration test, according to a number of investigators, is an in vitro correlate of delayed hypersensitivity (3, 4, 5, 12, 17), and it has been successfully applied in various clinical hypersensitivity states (1, 2, 6, 15). We therefore considered it of interest to investigate the effects of human dander on the in vitro migration of leukocytes from patients with atopic dermatitis.

MATERIALS AND METHODS

Subjects. 16 patients with atopic dermatitis, ranging in age from 14 to 34 years, were tested. The diagnosis was established on the basis of morphology and distribution of skin lesions. They had lichenified patches which involved the antecubital and popliteal fossae, and other predisposing areas. Twelve of the 16 patients had an immediate family history of respiratory atopy or eczema. The control group consisted of 10 healthy, non-atopic persons in the age range 20 to 42 years. They had never suffered from eczematous skin disease.

Human dander preparation. Dander from the scalp of healthy adults was defatted with acetone. 5 grams of the defatted dander was mixed in 50 ml of phosphate-buffered

saline (pH 8.0) and homogenized in a glass homogenizer. After the addition of 450 ml of the buffered saline, the homogenate was shaken at 4°C for 48 hours, and centrifuged at 12 000 rpm for 15 minutes. The extract was dialysed against distilled water. Since the activity of the extract with regard to skin reactions had been found to remain in the supernatant, the sediment was discarded. The supernatant was dried by lyophilization and used throughout the study.

Leukocyte migration test. The technique described by Sorborg & Bendixen (13) was followed, but with minor modifications. Heparinized blood (15-20 ml) was obtained from a cubital vein. White blood cells were harvested after dextran sedimentation and washed four times by serial centrifugation with Hanks' balanced salt solution. A 10% packed cell volume in TC-199 medium was then prepared. The cell suspension was aspirated into capillary tubes. One end of each tube was sealed with silicone, followed by centrifugation at 500 rpm for 5 minutes. The tubes were then cut just below the cell-fluid interface and the cell-containing part of each tube was fixed in a tissue culture chamber with silicone. Two control cultures were filled with TC-199 medium containing 100 µg of streptomycin per ml, and the other duplicate cultures were filled with the medium to which dander extract (600 µg protein per ml) was added. The cultures were incubated for 24 hours at 37°C and then the area over which the cells had migrated was projected through a projection microscope and traced on section paper. The area on the paper was measured by counting the sections. The quantitative value of the migration was calculated as the average value of two identically treated cultures. If, within one such pair of cultures, the variation from one migration area to another differed by more than 10 %, the test was discarded. The results were expressed as a migration index, which is the mean area of migration in chambers containing dander extract, divided by the mean area of migration in chambers without the extract.

Preliminary studies in which the protein concentration of the medium containing dander extract was 60, 150, 300, and 600 µg per ml established that the last of them gave the most consistent inhibition of migration of leukocytes from patients with atopic dermatitis. At this concentration, however, the migration of leukocytes from normal subjects was scarcely inhibited. This concentration was therefore used for the present study.

Skin test. After the leukocyte migration test had been carried out, skin tests were performed by the intracutaneous injection of 0.1 ml of a saline solution of the dander extract (10 µg protein per ml). The reactions were read after 48 hours.

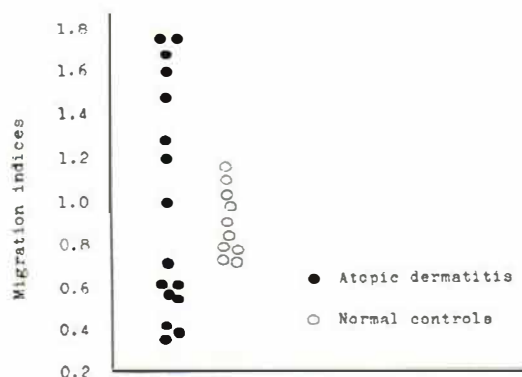


Fig. 1. Results of the leukocyte migration test using human dander as the antigen in 16 patients with atopic dermatitis and in 10 normal controls.

RESULTS

Fig. 1 shows the distribution of the migration indices of 16 patients with atopic dermatitis and 10 normal controls. When the indices of the atopic dermatitis patients are regarded as a group, the mean value is 1.07 ± 0.57 standard deviation, while the mean value of the controls is 0.96 ± 0.17 S.D. This difference in the standard deviation indicates that the observations from the patients with atopic dermatitis and the controls form two significantly different populations ($f = 10.58, p < 0.01$).

Migration indices below the lower limit of the normal range (mean -2 S.D.) were observed in 5 of the 16 patients with atopic dermatitis and indices over the upper limit of the normal range (mean $+2$ S.D.) in 6. Values in the remaining 5 patients fell within the normal range.

The relationship between leukocyte migration

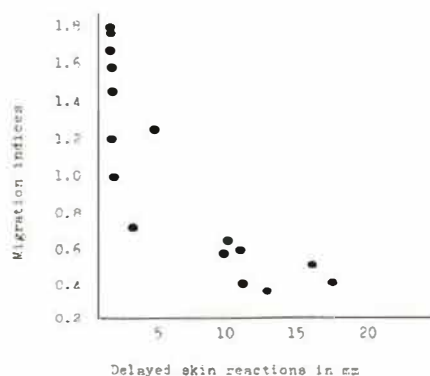


Fig. 2. The relationship between the delayed skin reaction and the migration index in patients with atopic dermatitis.

indices and delayed intracutaneous reaction to human dander in patients with atopic dermatitis is given in Fig. 2. It can be seen that inhibition of migration was elicited in those cases where delayed skin reactions to the dander were strongly positive, while stimulation of migration was observed in the patients with weak skin reaction.

DISCUSSION

We have previously reported that positive delayed intracutaneous reactions to human dander are more prevalent in patients with atopic dermatitis than in those with other dermatoses, or normal controls (16). This reaction grossly and histologically resembles the tuberculin reaction which is a manifestation of delayed hypersensitivity.

By using the leukocyte migration test, this study demonstrates that human dander induces abnormal migration indices in 11 out of 16 (68%) patients with atopic dermatitis, inhibition of migration being found in 5 and stimulation in 6. According to Søborg (14), it is necessary to use subtoxic antigen concentration to ensure inhibition of migration in all sensitive cases, and if the antigen concentration is fixed at a relatively low level, the results will depend upon the degree of sensitivity in such a way that high sensitivities induce inhibition and low sensitivities stimulation. In the present study, the limitation of the human dander source and the crude method of antigen preparation (although the protein concentration employed was considerably high, the preparation might contain a large amount of proteins without antigenic property) did not allow the use of an antigen concentration as high as desirable. This may account for the occurrence of inhibition as well as stimulation of migration in the present study. It is worthy of note that, at the antigen concentration employed, inhibition of migration could be elicited only in those cases where delayed skin reactions to the dander were strongly positive.

The migration of leukocytes from normal controls was scarcely affected by the dander.

Thus, it would appear from these *in vivo* intracutaneous and *in vitro* leukocyte migration responses that some patients with atopic dermatitis exhibit delayed hypersensitivity to components of human dander.

It remains to be determined whether the delayed hypersensitivity to human dander represents merely a superimposed sensitization due to the direct con-

tact between the dander and an already damaged skin of the patients, or whether the hypersensitivity has any relationship to the pathogenesis of the disorder.

REFERENCES

1. Becker, F. W., Krull, P., Deicher, H. & Kalden, J. R.: Leucocyte-migration test in sarcoidosis. *Lancet* **1**: 120, 1972.
2. Bendixen, G.: Organ-specific inhibition of the in vitro migration of leucocytes in human glomerulonephritis. *Acta Med Scand* **184**: 99, 1968.
3. Budtz-Jørgensen, E.: Delayed hypersensitivity to *Candida albicans* in man. Demonstration in vitro: the capillary tube migration test. *Acta Allergol (Kbh)* **27**: 41, 1972.
4. Clausen, J. E. & Søborg, M.: In vitro detection of tuberculin hypersensitivity in man. Specific migration inhibition of white blood cells from tuberculin-positive persons. *Acta Med Scand* **186**: 227, 1969.
5. Clausen, J. E.: Polymorphonuclear leucocytes in the specific antigen-induced inhibition of the in vitro migration of human peripheral leucocytes. *Acta Med Scand* **188**: 59, 1970.
6. Eddelston, A. L. W. F., Smith, M. G. M., Mitchell, C. G. & Williams, R.: Application of the leucocyte migration test to the diagnosis of rejection after liver transplantation. *Transplantation* **12**: 11, 1971.
7. Lobitz, W. C., Honeyman, J. F. & Winkler, N. W.: Suppressed cell-mediated immunity in two adults with atopic dermatitis. *Br J Dermatol* **86**: 317, 1972.
8. Palacios, J., Fuller, E. W. & Blaylock, W. K.: Immunological capabilities of patients with atopic dermatitis. *J Invest Dermatol* **47**: 484, 1966.
9. Rajka, G.: Delayed dermal and epidermal reactivity in atopic dermatitis (prurigo Besnier). I. Delayed reactivity to bacterial and mold allergens. *Acta Dermatovener (Stockholm)* **47**: 158, 1967.
10. — Delayed dermal and epidermal reactivity in atopic dermatitis (prurigo Besnier). III. Further studies with bacterial and viral allergens. *Acta Dermatovener (Stockholm)* **48**: 186, 1968.
11. — Delayed reactivity to bacterial and viral extracts in different dermatoses. *Acta Dermatovener (Stockholm)* **50**: 281, 1970.
12. Søborg, M.: In vitro detection of cellular hypersensitivity in man. Specific migration inhibition of white blood cells from brucella positive persons. *Acta Med Scand* **182**: 167, 1967.
13. Søborg, M. & Bendixen, G.: Human lymphocyte migration as a parameter of hypersensitivity. *Acta Med Scand* **181**: 247, 1967.
14. Søborg, M.: In vitro migration of peripheral human leucocytes in cellular hypersensitivity. *Acta Med Scand* **184**: 135, 1968.
15. Thulin, H. & Zachariae, H.: The leukocyte migration test in chromium hypersensitivity. *J Invest Dermatol* **58**: 55, 1972.
16. Uehara, M. & Ofuji, S.: Delayed skin reactivity to human dander in atopic dermatitis. *Acta Dermatovener (Stockholm)* **49**: 294, 1969.
17. Wolfson, R. L., Maddison, S. E. & Kagan, I. G.: Migration inhibition of peripheral leucocytes in human schistosomiasis. *J Immunol* **109**: 123, 1972.

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