

B LYMPHOCYTES, T LYMPHOCYTES AND PHYTOHAEMAGGLUTININ RESPONSIVENESS IN ATOPIC DERMATITIS

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Abstract. The spontaneous binding of sheep erythrocytes to human lymphocytes with the formation of rosettes was used as a measure of thymus-derived lymphocytes and the rosette formation with complement-coated sheep erythrocytes as a measure of bursa-dependent lymphocytes in the peripheral blood of patients with atopic dermatitis. Both percentage proportion and the absolute number of thymus-derived lymphocytes were slightly but significantly reduced. In a few patients with atopic dermatitis the percentage and absolute number of bursa-dependent lymphocytes were increased. However, when all values were considered, no significant differences were demonstrated. The responsiveness of cultured lymphocytes to phytohaemagglutinin was normal when the culture medium contained foetal calf serum. The response of normal lymphocytes was not impaired by serum from patients with atopic dermatitis. However, the response was depressed when atopic dermatitis lymphocytes were cultured in medium containing autologous serum, indicating that a partial lymphocyte defect becomes manifest only in the presence of a serum factor, presumably IgE.

Key words: B lymphocytes; T lymphocytes; Lymphocyte transformation; Dermatitis atopic

Cell-mediated immunity may be defective in patients with atopic dermatitis. Lobitz et al. (6) described two patients with impaired response to phytohaemagglutinin (PHA), negative delayed-type skin reactions, lack of response to sensitisation with DNCB, and depletion of lymphocytes from paracortical areas of the lymph nodes. PHA responsiveness was restored in one of the patients when the atopic dermatitis improved during treatment.

It was the purpose of the present investigation to study whether impairment of cell-mediated immunity is a common finding in patients with atopic dermatitis.

The number of thymus-dependent lymphocytes was determined by their ability to bind sheep erythrocytes spontaneously (E-binding lymphocytes) and the bursa-dependent lymphocytes by a rosette method demonstrating receptors for activated com-

plement on the surface of the lymphocytes (EAC-binding lymphocytes). Furthermore the ability of the lymphocytes to respond to PHA in a standard culture medium was measured. The influence of serum factors was investigated by measuring the PHA response of normal lymphocytes in the presence of serum from patients with atopic dermatitis and that of atopic lymphocytes in the presence of autologous serum.

MATERIALS AND METHODS

Thirteen females and 6 males with atopic dermatitis were investigated. Their median age was 30 years, range 10-40. All atopic dermatitis patients over the age of 10 years, who were admitted to the department during the investigation period, were included in this study. However, since the patients were referred from dermatologists with private practice, there might be some selection—most likely of more severe cases. In 14 of the patients the onset of disease was within their first 2 years of life and in the remaining 5 the disease had lasted for 4 to 18 years. Erythema, induration and lichenification of large skin areas was found in all patients. Universal involvement was found in 14, whereas in 5 patients the lesions were somewhat more localized. 11 patients had enlarged lymph nodes in axillae and inguinal regions. 13 had positive skin tests for pollen and animal hair and 11 for house dust. IgE varied between 405 and 11 500 units with a median of 4 700 (Pharmacia Service Laboratorium. Normal values <500) (4).

The control material comprised 8 females and 7 males. 11 were laboratory staff members and 4 were non-hospitalized volunteers. Their median age was 30 years, range 11-47.

White blood cells were counted in a Bürker-Türk counting chamber. Differential count was made by counting 300 white cells and the absolute number of lymphocytes calculated.

For the demonstration of E and EAC binding cells, lymphocytes were isolated by centrifugation on a gradient of Ficoll and meglumine amidotriacetate (Urografin®) as previously described (1). This is a slight modification of the method originally described by Böyum (2). The lymphocytes were washed in Hanks buffered salt solution (HBSS) and

concentrated by centrifugation three times, and finally the concentration was adjusted to 4×10^6 lymphocytes/ml.

E and EAC binding lymphocytes were demonstrated by the method described by Stjernswärd et al. (9). Sheep erythrocytes, always obtained from the same sheep, were stored in Alsewer's solution and used within one week from bleeding. Before use the erythrocytes were washed twice in HBSS. For the detection of E binding cells the washed erythrocytes were adjusted to a 1.8% suspension and for EAC binding cells to a 5% suspension.

For the demonstration of E binding cells, 0.25 ml of the lymphocyte suspension was mixed with 0.25 ml of the washed sheep erythrocytes, incubated for 15 min at 37°C and then spun for 5 min at 200 g and incubated in ice overnight. The cells were then resuspended by gentle shaking for 30 sec. One drop was mounted on a glass slide, covered by a coverslip and sealed with silicone grease. 200 lymphocytes were counted in a phase contrast microscope, using a standardized reading technique. Lymphocytes binding 3 or more erythrocytes were counted as rosettes. Results were expressed as percentage E binding cells (E%) and as absolute number of E binding cells per μ l (E/ μ l).

For the demonstration of EAC binding cells, 5 ml of washed sheep erythrocytes was mixed with 5 ml of amboceptor 1/2000 in HBSS (anti sheep hemolysin, Behringwerke AG) and incubated for 30 min at 37°C, then washed three times and resuspended in 5 ml of HBSS. 5 ml of fresh human serum 1/20 in HBSS was added and the suspension incubated for another 30 min at 37°C. Finally the cells were washed three times and adjusted to a 1% suspension. 0.25 ml of this suspension was mixed with 0.25 ml of the lymphocyte suspension, centrifuged at 200 g for 2 min and left at room temperature. After 15 min the cells were resuspended by mixing on a whirl-mixer and counted as described above. Results were expressed as percentage EAC binding cells (EAC%) and as absolute number of EAC binding cells per μ l (EAC/ μ l).

For PHA stimulation, buffy coat leucocytes were separated by dextran sedimentation of the erythrocytes. 8 parts of heparinized venous blood were mixed with 2 parts of dextran (mol. wt. 75 000; 3% in 0.9% saline) and allowed to sediment for 60 min at 37°C. The leucocytes from the supernatant were washed three times with HBSS and resuspended in medium 199 (Difco Laboratories) supplemented with 0.015 ml of glutamine (Flow Laboratories, 200 mmol) 0.75 ml of non-essential amino acids (Flow Laboratories, 100 $\times$) and 30 ml of foetal calf or human serum per 100 ml of medium 199. The lymphocyte concentration was adjusted to 1×10^6 /ml counting in a phase contrast microscope and further dilution with culture medium.

1.5 ml cultures were set up in triplicate and with appropriate controls. 30 μ l of PHA (Wellcome, batch 5089) was added to each culture. Cultures were gassed with atmospheric air containing 5% CO₂ and kept in a water bath at 37°C. After 48 h the cultures were pulsed for 3 h with 0.75 μ Ci of methyl-³H-thymidine (The Radiochemical Centre Amersham, 19 Ci/mmol). Then the cells were isolated on glass filter discs according to the method described by Freiestlebens-Sørensen et al. (3). The filters were successively treated with 60 ml of 0.9% saline, 40 ml of ice-cold trichloroacetic acid and 40 ml of methanol. The dried filters were incubated with 0.3 ml of hyamine (Packard) for 24 h at 37°C and finally

Table I. Absolute numbers of lymphocytes, percentage of E binding (thymus-dependent) and EAC binding (bursa-dependent) lymphocytes and absolute numbers of E and EAC binding lymphocytes in controls and in patients with atopic dermatitis

	Controls		Atopic dermatitis	
	Median	Range	Median	Range
Lymphocytes/ μ l	2 081	1 176-3 289	1 843	1 145-3 186
E%	60	49-70	51	34-68
E/ μ l	1 240	538-2 039	994	550-1 497
EAC%	28	17-35	28	18-48
EAC/ μ l	585	301-1 085	589	226-1 211

10 ml of scintillator (0.05 g POPOP and 4 g PPO in 1 litre of toluene). Liquid scintillation was performed using a Packard Tri-carb model 3385. Results were expressed as counts per minute.

Effects of serum factors were investigated in 'cross-over' experiments. Lymphocytes from patients and from appropriate control individuals were cultured in medium in which foetal calf serum was replaced by homologous and autologous serum respectively.

Wilcoxon's test for two continuous samples were employed for the statistical analysis.

RESULTS

Table I shows the absolute lymphocyte count and subpopulations in patients with atopic dermatitis, and in controls. Lymphocyte counts show a slightly, though not significantly, lower median value in patients than in controls. The median value of E% and E/ μ l are lower in the patient group than in the control group. The difference is not highly significant (Wilcoxon test: $0.025 > p > 0.01$ and $0.05 > p > 0.025$ respectively).

In Figs. 1 and 2 the lymphocyte subpopulations are illustrated diagrammatically. There is a considerable overlap between the two groups. In the atopic dermatitis group two E% are markedly lower than the other values in this group. Clinically there seem to be no major differences between those 2 patients and all the others. In the same group five values of E/ μ l as well as one in the control group are clearly lower than the rest of these values. These 5 patients had a relatively low E% and also a low absolute lymphocyte count.

EAC binding capacity was investigated in 17 of the patients. EAC% varies considerably in patients with atopic dermatitis and so does the absolute

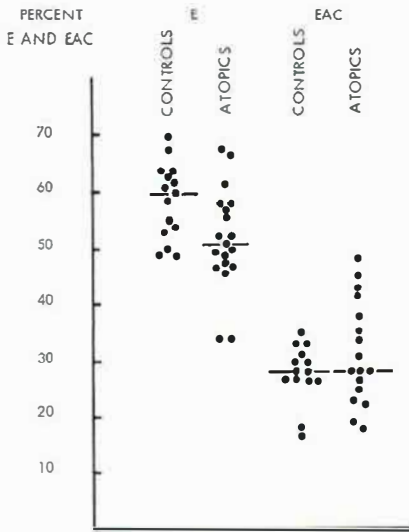


Fig. 1. Percentage of E binding (thymus-dependent) and EAC binding (bursa-dependent) lymphocytes in controls and in patients with atopic dermatitis.

number of EAC binding lymphocytes. Five patients had a remarkable high EAC%, though the difference between patients and controls is not significant.

Table II gives the median values of the PHA responses in 18 of the patients with atopic dermatitis and in 12 of the controls. Fig. 3 shows these results in diagram form. The PHA responses of the patients did not differ from that of the controls when

Table II. *Phytohaemagglutinin responsiveness of cultured lymphocytes from controls and patients with atopic dermatitis, expressed as counts $\times 10^6$ per min*

	Controls		Atopic dermatitis	
	Median	Range	Median	Range
Medium 199 + foetal calf serum	192	76-336	177	85-282
Medium 199 + homologous serum	166	75-253	157	58-318
Medium 199 + autologous serum	155	58-273	105	44-226

leucocytes are cultured in a medium containing foetal calf serum. No inhibition of the response was demonstrated when normal leucocytes were cultured in the presence of serum from patients with atopic dermatitis, when compared with patient leucocytes cultured in the presence of serum from the controls.

Leucocytes from 12 of the controls and 13 patients were cultured in media containing autologous serum. Under these conditions, lymphocytes from the atopic dermatitis patients showed significantly lower responses to PHA than did lymphocytes from the controls ($0.01 > p > 0.005$).

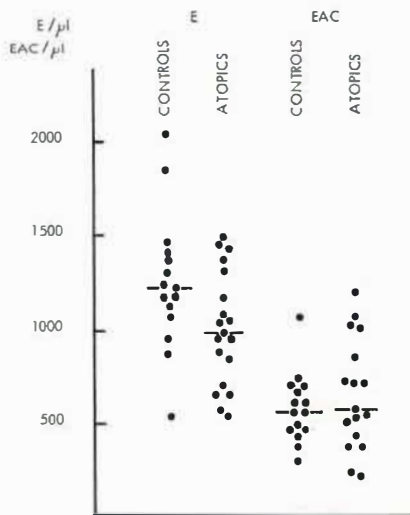


Fig. 2. Absolute numbers of E binding (thymus-dependent) and EAC binding (bursa-dependent) lymphocytes in controls and in patients with atopic dermatitis.

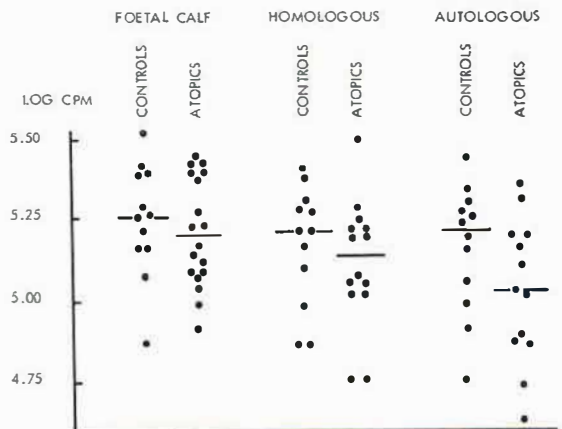


Fig. 3. Phytohaemagglutinin responsiveness of cultured lymphocytes in controls and in patients with atopic dermatitis. Lymphocytes were cultured in medium 199 containing 30% foetal calf serum, in medium 199 containing 30% homologous serum (normal lymphocytes in the presence of serum from patients with atopic dermatitis and vice versa) and finally in medium 199 containing 30% autologous serum.

There is no correlation between either the levels of IgE on the one hand and E%, EAC% or PHA responsiveness on the other, in patients with atopic dermatitis, or between PHA responsiveness of atopic lymphocytes and the concentration of IgE in autologous sera used as additive in the culture media.

DISCUSSION

The present investigation demonstrates a slight reduction in the percentage and absolute number of thymus-dependent lymphocytes in a group of patients with atopic dermatitis, mainly adults with persistent extensive skin lesions, lymph node enlargement and elevated serum IgE levels.

Sheep erythrocytes were used as marker of the lymphocytes. The method detects thymus-dependent lymphocytes with a high degree of certainty (5).

Transformation of lymphocytes into blast-like cells induced by PHA is supposed to be an *in vitro* correlate of the function mainly of thymus-dependent lymphocytes. A small proportion of these cells in lymphocyte suspensions exposed to PHA presumably results in a decrease of the response to the mitogen. The slight reduction of the percentage of thymus-dependent lymphocytes in our patients with atopic dermatitis did not lead to a measurable decrease of PHA responsiveness when the lymphocytes were cultured in a medium containing foetal calf serum. However, the lymphocytes of these patients showed a significantly lower response when cultured in medium containing autologous serum. Serum from the patients with atopic dermatitis did not contain, at least not in measurable amounts, factors with inhibitory effect on the PHA responsiveness of normal lymphocytes.

One plausible explanation of the findings mentioned above would be, that a partial defect in the function of thymus-dependent lymphocytes in atopic dermatitis became manifest only in the presence of IgE or other factors found in the serum of these patients.

Lobitz et al. (6) could demonstrate diminished PHA responsiveness in 2 patients with lifelong severe atopic dermatitis, even when the lymphocytes were cultured in medium containing foetal calf serum. In one of the patients the response returned to normal when the disease improved during treatment. In a recent study by Schöpf & Böhringer (8) lymphocytes were cultured in medium containing autologous serum and stimulated either by PHA or by oxym-

curisalicic acid, another unspecific T-cell mitogen. In contrast to Lobitz et al., these authors found relatively increased responsiveness in atopic dermatitis. However, the ages of the controls were considerably higher than that of their atopic dermatitis patients, and many of the patients seem to have less severe symptoms than the patients included in the present study. Three of the patients described by Schöpf & Böhringer showed signs of worsening of the disease during the past few weeks before the lymphocyte culture was made. A significantly weaker response to oxymcurisalicic acid was found in these patients, as compared with patients with stable disease.

Thus, there is a considerable discrepancy between the findings in the different investigations, though this might be the result merely of differences in extension and activity of the disease rather than differences in the techniques employed.

Complement receptors on the cell surface were used for the demonstration of bursa-dependent lymphocytes (7). In 5 out of 17 patients with atopic dermatitis a high proportion of complement receptor bearing lymphocytes was demonstrated. This phenomenon was not further investigated. In the cases reported by Lobitz et al., a relatively high percentage of small and medium-sized lymphocytes in the peripheral blood displayed anti IgE fluorescence. This might in fact be the explanation of the findings of some relatively high EAC% (bursa-dependent lymphocytes) in the present investigation.

Our study confirms that impairment of the cell-mediated immune function may be a feature of atopic dermatitis. We have further shown that the number of circulating thymus-dependent lymphocytes may be reduced in this condition. In some instances the functional impairment seems to be manifest only when serum factors are present. It may be that this impairment is caused by a high IgE level, since the impairment is to some degree correlated to the extension or activity of the disease.

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REFERENCES

1. Andersen, E.: Depletion of thymus dependent lymphocytes in Hodgkin's disease. *Scand J Haemat* 12: 263, 1974.
2. Böyum, A.: Separation of leucocytes from blood and bone marrow. *Scand J Clin Lab Invest, Suppl.* 97: 7, 1968.

3. Freiesleben-Sørensen, S., Andersen, V. & Giese, J.: A rapid method for quantitation of the incorporation of ³H-thymidine by lymphocytes in vitro. *Acta Path Microbiol Scand* 75: 508, 1969.
4. Johansson, S. G. O., Bennich, H. & Wide, L.: A new class of immunoglobulin in human serum. *Immunology* 14: 265, 1968.
5. Jondal, M., Holm, G. & Wigsell, H.: Surface markers on T and B lymphocytes. I. A large population of lymphocytes forming non immune rosettes with sheep erythrocytes. *J Exp Med* 136: 207, 1972.
6. 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.
7. Michlmayr, G. & Huber, H.: Receptor sites for complement on certain human peripheral blood lymphocytes. *J Immunol* 105: 670, 1970.
8. Schöpf, E. & Böhringer, D. Zell-vermittelte Immunität bei Neurodermitis atopica. *Hautarzt* 25: 420, 1974.
9. Stjernswärd, J., Jondal, M., Vanky, F., Wigsell, H. & Sealy, R.: Lymphopenia and change in distribution of human

B and T lymphocytes in peripheral blood induced by irradiation for mammary carcinoma. *Lancet* *i*: 1352, 1972.

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ADDENDUM

Since the termination of the present study, Luckasen et al. have published results indicating a moderate reduction of T lymphocytes in patients with atopic dermatitis (Luckasen, J. R., Sabad, A., Goltz, R. W. & Kersey, J. H.: T and B lymphocytes in atopic eczema. *Arch Dermatol* 110: 375, 1974).