

AN IN VITRO STUDY OF DEPRESSED CELL-MEDIATED IMMUNITY AND OF T AND B LYMPHOCYTES IN ATOPIC DERMATITIS

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Abstract. Lymphocyte transformation tests, E binding (T) rosette assay and immunofluorescent preparations of B cells were studied in patients with atopic dermatitis, and also in healthy controls. Most of the patients were found to have high levels of IgE in serum. The patients were studied both during severe bouts of dermatitis and also when the dermatitis was almost healed. Lymphocyte transformation tests showed that patients were hyporeactive to PPD and herpes simplex antigen in vitro, both during periods of severe dermatitis and also when the dermatitis was in remission. The response to PHA in the patients was normal in vitro. No factors which could reduce cell-mediated immunity in vitro were found in patients' sera. A decreased number of T lymphocytes and a slight increase in the number of immunoglobulin-bearing lymphocytes (B cells) were demonstrated in the patients, both during remission and during recurrence of severe dermatitis. In 3 of 8 patients, increased numbers of IgE-bearing lymphocytes were found to be present when the patients had severe dermatitis. A possible correlation between high serum levels of IgE and depressed cell-mediated immunity in patients with atopic dermatitis is discussed.

Key words: Dermatitis atopic; Cell-mediated immunity; Lymphocyte transformation; T lymphocytes; B lymphocytes

The pathogenesis of atopic dermatitis (AD) is still unclear. Immunological disturbances such as high IgE levels in the serum are often present (18, 19). Hyporeactivity in cell-mediated immunity has also been demonstrated in AD, with both in vivo (6, 10, 13, 18) and in vitro (6, 10, 12, 18) methods.

Thestrup-Pedersen et al. (17) have shown that the response of cultured lymphocytes to concanavalin A, pokeweed mitogen, and PPD was depressed in patients with severe AD when the reaction took place in the presence of autologous plasma.

Andersen et al. (1) found normal responses to PHA in foetal calf serum, but depressed responses when lymphocytes from patients with AD were cultured in medium containing autologous serum. However, Grove et al. (4), examining patients with AD, found normal stimulation with PHA, both with foetal calf serum and with autologous plasma, and Schöpf et al. (15) found increased lymphocytic ³H-thymidine uptake when patients with AD were tested both with and without autologous serum. This was valid both for spontaneous stimulation and after stimulation with PHA. McGeady et al. (12) found normal responsiveness to PHA but depressed responses to the mitogens con A and pokeweed mitogen, as well as to candida antigen, and the results were the same whether the media were supplemented with autologous or normal homologous plasma. Wüthrich (18) demonstrated hyporeactivity to candida antigen in vitro in 3 of 6 patients with AD in comparison with 1 in 15 controls, yet PHA stimulation was normal.

In a previous study (6) it was shown by means of lymphocyte transformation tests (LTT) that patients with AD were hyporeactive to PPD and herpes simplex antigen. This was most prominent in those patients having high IgE levels. However, no difference was found in this investigation between patients and controls with regard to their response to PHA in vitro. There have been reports of both low (1, 5, 11, 12, 14) and normal (2, 4) numbers of T cells in the peripheral blood of patients with AD, and B lymphocytes have been reported to be both increased in number (2, 3, 12) and normal (1, 4, 11). Cormane et al. (3) and Carapeto et al. (2) found increased numbers of IgE-bearing lymphocytes

Table 1. Results of lymphocyte transformation tests in patients with atopic dermatitis and controls

Activity of the dermatitis ...	Patients						Controls		
	+++			(+)			0		
	Mean value			Mean value			Mean value		
	Counts	Ratios	n	Counts	Ratios	n	Counts	Ratios	n
Without stimulation	2 932	—	22	3 662	—	22	3 369	—	14
PHA	72 000	37	16	66 000	25	16	62 000	30	10
PPD	2 780 ^a	2.7 ^c	22	4 273 ^d	3.0 ^d	22	10 199	5.5	14
Herpes simplex antigen	2 253 ^a	2.6 ^b	19	3 982 ^d	3.0 ^d	19	12 528	6.5	14

^a $p < 0.002$. ^b $p < 0.01$. ^c $p < 0.02$. ^d $p < 0.05$.

(7–18 %) in patients with AD, whereas McGeady et al. (12) found normal numbers.

Thus controversy exists concerning the numbers of T and B cells in the peripheral blood, the results of T cell mitogen stimulation and also the role of autologous plasma in LTT in this patient group. There seems, however, to be a tendency to hyporeactivity in the cell-mediated immunity when stimulation is performed with antigens, both in vivo (6, 10, 13, 18) and in vitro (6, 12, 18). Too little is known of the immunological status in atopic subjects to say whether this cell-mediated immunity defect is primary or secondary.

There are many possible explanations for the contradictory results of mitogen stimulation and lymphocyte counting. In addition to the variety of laboratory techniques, the selection of patients is of importance. The severity of the dermatitis, the IgE level in the serum, secondary infection in the skin and the prevailing treatment of the dermatitis are all factors that may influence the results of the in vitro tests.

The purpose of this investigation was to observe patients with AD and study LTT and the numbers of T and B cells both when they had exacerbations of severe dermatitis and when the dermatitis was almost or completely healed. In order to obtain more comparable test results, the lymphocytes were frozen and stored, and the two cell samples from each patient were investigated simultaneously with one control sample.

MATERIAL AND METHODS

Patients and controls

The patient material was obtained from the Department of Dermatology, Södersjukhuset, Stockholm. Only patients

with a tendency to severe dermatitis and who professed freedom from eczema at least once a year were included in the study. There were 16 women and 6 men, mean age 25 years. The controls were healthy volunteers, 8 women and 7 men, mean age 28 years. (Not all the laboratory tests were performed on all the patients and controls.) Tests were conducted on the patients both during bouts of severe dermatitis and also during quiescent periods of eczema activity.

Activity grading

Severe dermatitis (+++) = active dermatitis affecting more than 1/3 of the body surface. Low eczema activity (+) = active dermatitis on less than 5% of the body surface, or no dermatitis. Only a few patients were totally free of dermatitis during the observation period. Thus most patients had slight eczema (such as some eczema in the bends of the knees or slight eczema on the hands) when the tests for low eczema activity were taken. Four patients not satisfying the activity grading criteria both with regard to severe dermatitis and to low eczema activity during the year they were studied were excluded.

One of the patients had suffered from asthma, and 9 of the 22 patients had experienced rhinitis at some time during recent years. The IgE level was more than 1 000 U/ml in 18 of the 22 patients.

None of the patients had used corticosteroid tablets during the year prior to the tests. During periods of low eczema activity, the patients had not displayed any clinical signs of secondary infection and had not used potent steroids topically during the weeks prior to that test.

METHODS

The lymphocytes were separated by the Ficoll Isopaque method as described previously (6). Freezing, storage in liquid nitrogen and thawing of the lymphocytes were performed at the Immunobiology Laboratory, St Görans Hospital, Stockholm. Purified lymphocytes in Parker 199 with 10% heat-inactivated human AB serum were mixed with an equal volume of 20% dimethylsulphoxide (DMSO). One-ml samples were frozen in a multiple-freezing cartridge in liquid nitrogen, where the cell

Table II. Results of lymphocyte transformation tests with autologous serum in controls and in patients with atopic dermatitis expressed as

Activity of the dermatitis ...	controls/min with autologous serum				counts/min without autologous serum							
	Controls				Patients							
	0				(+)				+ + +			
	0 stim	PHA	PPD	HS ^a	0 stim	PHA	PPD	HS ^a	0 stim	PHA	PPD	HS ^a
Heat-inactivated autologous serum	0.80	0.30	1.00	0.40	1.00	0.30	0.80	1.36	1.00	0.30	0.55	0.90
	—	0.63	1.38	0.95	0.86	0.62	1.03	1.19	0.63	0.54	0.76	1.25
	0.69	0.89	0.67	1.02	0.53	0.37	1.00	2.25	0.92	0.69	1.12	1.76
	0.76	—	2.19	1.10	0.53	0.18	2.85	—	0.56	0.16	1.19	0.89
	1.38	0.20	1.61	2.01	1.28	—	1.37	2.20	1.13	1.15	1.35	1.14
	1.15	0.40	1.36	0.91	0.65	0.82	1.02	1.05	1.16	—	1.01	1.45
	—	—	—	—	0.81	0.38	0.74	0.66	0.58	0.20	2.75	1.01
	—	—	—	—	0.79	0.21	1.01	0.60	0.60	0.22	1.35	1.31
	—	—	—	—	0.84	0.46	1.31	1.30	0.88	0.66	1.10	1.21
	—	—	—	—	—	—	—	—	—	—	—	—
Not-heat-inactivated autologous serum	1.00	0.75	1.23	1.08	1.08	1.04	1.07	1.96	1.20	0.76	1.80	1.94
	0.62	0.48	1.58	1.07	—	—	—	—	2.2	0.58	1.58	1.21
	—	0.75	0.60	0.50	—	0.50	0.85	1.02	—	—	1.24	0.70
	0.80	0.30	1.00	0.40	1.00	0.30	0.80	1.36	1.00	0.30	0.55	0.90
	1.10	0.90	1.30	1.10	0.70	1.20	1.20	1.30	1.20	0.80	1.60	0.90
	—	—	—	—	—	—	—	—	1.00	0.80	2.00	2.00
Median value	0.80	0.56	1.30	1.02	0.83	0.42	1.02	1.30	1.00	0.56	1.22	1.18
	—	—	—	—	—	—	—	—	0.57	0.36	0.72	1.15

^a Herpes simplex antigen.

reached minus 196°C in 20 min. For thawing, the test tubes were kept for 5 min in a 37°C water bath. The cells were washed three times in HT with 0.1% gelatin.

The two lymphocyte samples from one patient (or the four samples from two patients) and one sample from a control were thawed at the same time, whereafter LTT was performed and T and B cells were counted.

Lymphocyte transformation tests (LTT)

LTT was performed by the method described previously (6). PHA (10 µg/10⁶ cells/ml) was used as mitogen for T lymphocytes in the case of 16 patients and 10 controls. PPD (1 µg/10⁶ cells/ml) and herpes simplex antigen diluted 1:20 were used for antigen stimulation tests. Twenty-two patients and 14 controls were tested with PPD and 19 patients and 14 controls were tested with herpes simplex antigen. Lymphocytes from patients and controls were also cultured in medium which, besides 10% human AB serum, also contained 10% autologous serum. The autologous sera were heat-inactivated in 10 patients and 6 controls in order to destroy the IgE.

E-binding (T) rosette assay

The capacity of human peripheral lymphocytes to bind sheep red blood cells (SRBC) was used as a marker for T cells. The method used was essentially that described by Jondal et al. (8), in which 0.5 ml of a 5% suspension of several times washed SRBC in undiluted, heat-inactivated foetal calf serum, absorbed with SRBC, was added to 0.5 ml of a cell suspension containing 5×10⁶ lymphocytes

diluted in BSS. The cells were incubated for 30 min at 37°C, and the mixture was then spun at 4°C for 5 min at 800 rev/min. The cells were kept on ice overnight and thereafter carefully resuspended and counted. All lymphocytes which had absorbed more than two SRBC were counted as rosettes.

Immunofluorescence staining

The lymphocytes were washed several times in BSS and thereafter 5×10⁶ lymphocytes were incubated for 30 min at room temperature with 0.05 ml of a fluorescein-labelled polyvalent anti-human immunoglobulin serum (Anti-human IG SHO 71410 SBL, Sweden). In 8 patients and 8 controls, incubation was also performed with a fluorescein-labelled antihuman IgE serum (Behringwerke AG, Germany). Thereafter, the cells were washed three times in BSS, suspended in the same volume of BSS, and kept on ice until the living fluorescent cells were counted in a Leitz fluorescence microscope.

Statistics

Students *t*-test was used in the statistical analysis. The mean values of counts and ratios were calculated subsequent to logarithmic transformation of the values. To make it possible to perform statistical analysis of the logarithmically transformed count value increases, low values (=500 cpm) were awarded to tubes with no stimulation at all, so that the problem with log₁₀ could be overcome.

Table III. Results of E-binding (T) rosette assay and immunofluorescence studies with polyvalent anti-human immunoglobulin serum, expressed as mean values

Activity of the dermatitis ...	Controls		Patients			
	0		(+)		+++	
	M	n	M	n	M	n
T rosettes, %	59.3	15	51.2 ^b	15	52.6 ^c	15
Immunofluorescence, %	16.1	12	18.2 ^c	12	19.9 ^a	12

^a $p < 0.01$. ^b $p < 0.02$. ^c $p < 0.05$.

RESULTS

About 25 % of the cells were lost due to the freezing and the washings after thawing.

Results of lymphocyte transformation tests

There was a tendency towards lower stimulation ratios when LTT was done with frozen, vis-à-vis not-frozen, lymphocytes. The mean values of the ³H-thymidine uptake in cultures without antigens or mitogens did not differ statistically in the different groups (Table I).

In the tests involving PHA stimulation (Table I), the stimulation tended to be weaker in the patients at times of low eczema activity than on those occasions when they had severe dermatitis. The differences, however, were not statistically significant, and neither were any statistical differences found in the mean values of the patients and the controls.

The measurements of the ³H-thymidine uptake into blood lymphocytes after stimulation with PPD are documented in Table I.

In comparison with the controls, statistical analysis of the logarithmically transformed values showed lower mean values in the patients both when they had low eczema activity (counts and ratios $p < 0.05$) and when they had severe dermatitis (counts $p < 0.002$ and ratios $p < 0.02$). No significant differences were found between the results in patients when they had low eczema activity or when they had severe dermatitis.

After stimulation with herpes simplex antigen (Table I) the results were similar to those observed with PPD in vitro. Thus lower stimulations were seen in the patients than in the controls, both when the patients had low eczema activity (ratios and counts $p < 0.05$) and when they had severe dermatitis (counts $p < 0.002$, ratios $p < 0.01$), but no statistically significant differences were observed

between the patients when they had low eczema activity and when they had severe dermatitis.

The results of LTT with autologous serum are set out in Table II, showing that the PHA response in tubes with autologous serum was reduced by about 50 % in both patients and controls. When stimulation was performed with antigens (PPD and herpes simplex) there was a slight tendency towards increased transformation in most of the tubes containing autologous serum, in both patients and controls. The tendencies were the same whether the autologous sera were heat-inactivated or not.

Results of the E-binding (T) rosette assay

The results of the T rosette tests are given in Table III. The patients showed statistically significant lower numbers of T rosette formations than in the controls, but there was no significant difference between a patient during a bout of severe dermatitis and when in remission.

Results of the immunofluorescence staining

The results of immunofluorescence staining with polyvalent anti-human immunoglobulin serum are presented in Table III. The patients showed a slight but statistically significant increase in the number of immunoglobulin-bearing cells. There was no statistically significant difference between the patients when the eczema was inactive and when it was active.

Tests for IgE-bearing lymphocytes were performed in 8 patients and 8 controls. Values between 0.5 % and 3 % were obtained in both controls and patients with the exception of 3 patients. These 3 had 12 %, 9 % and 6 % IgE-bearing lymphocytes when they had severe dermatitis, and 7 %, <3 % and <3 % respectively when they had low eczema activity.

DISCUSSION

Lobitz et al. (10) found hyporeactivity to PHA *in vitro* in two patients with severe atopic dermatitis, and Schöpf et al. (14) found increased lymphocyte transformation in patients with atopic dermatitis after stimulation with PHA. However, most authors (1, 4, 6, 12, 18) have reported normal responses to PHA when lymphocytes from patients with atopic dermatitis were cultured in media containing foetal calf serum or human AB serum. Similarly, the results of the present study also indicate that patients with AD usually show a normal response to PHA *in vitro* both when the dermatitis is active and when it is inactive.

The results of LTT with antigens (PPD and herpes simplex antigen) confirm earlier investigations (6, 12, 18) which have shown that the lymphocytes of patients with AD are hyporeactive when they are stimulated with "T-cell antigens". The investigation has also shown that, in patients with a tendency to periods of severe AD, the hyporeactivity in the cell-mediated immune system remains even when the dermatitis is completely or almost completely healed. Some patients had had low eczema activity only 2 weeks before the tests were done, whereas other patients had been in remission for more than 2 months. However, it is not possible to say from this investigation, if or when normalization of the cell-mediated immune system takes place, if the patients have been free from eczema for a long time.

When the patients had low eczema activity they had no signs of secondary infection and they had not used potent corticosteroids topically, during the weeks prior to the test. Thus it is unlikely that prevailing secondary infection or concurrent treatment could be responsible for the hyporeactivity in the cellular immune system. Leguit et al. (9) have shown, by means of LTT, that in patients with burns the specific response to antigens is initially low but returns to a normal level in about a month. Thus it is unlikely that the cell-mediated hyporeactivity in AD is only secondary to a spread injury to the skin.

Tada et al. (16) have shown that T lymphocytes have an important function in regulation (suppression) of reaginic antibodies in rats. In a previous study (6) it has been shown that the cell-mediated hyporeactivity in patients with AD is correlated to the serum levels of IgE and this supports the hypothesis that excessive production of IgE anti-

bodies may be related to defective thymus-derived immune function. Öhman et al. (19) found no pronounced variation in the serum IgE levels in patients with AD monitored during the course of a year, although the dermatitis in most cases improved during the summer.

Normalization of the IgE levels seem to occur first when the eczema has been in remission for a long time (7, 18). Most of the patients in this study had high IgE levels and the findings support the hypothesis that the hyporeactivity of the T cells correlates with high IgE levels, and that normalization of the cell-mediated immunity may occur slowly after the eczema has healed and thus could parallel the slow normalization of the IgE levels.

The LTT with autologous sera was performed to study whether sera from patients with AD contained factors (heat-stable or heat-labile, such as IgE) which suppressed *in vitro* T cell stimulation in AD patients. No such factors could be demonstrated with the method used. The response to PHA stimulation with autologous serum was reduced to the same extent in both patients and controls. The reason for this might be that some of the PHA was absorbed by increasing the serum from 10% to 20%.

There was a tendency towards an increased response to antigens both in patients and controls when autologous serum was added to the cultures, and this could have improved the conditions for the lymphocytes when the serum was increased from 10% to 20%.

The results of the T rosette assay and the immunofluorescence staining confirm earlier investigations that patients with AD have a reduced number of lymphocytes with receptors for SRBC (1, 5, 11, 12, 14) and an increased number of immunoglobulin-bearing lymphocytes (2, 3, 12). These disturbances of the lymphocytes do not seem to normalize immediately upon healing of the eczema.

In this study, 3 of 8 patients showed increased numbers of IgE-bearing lymphocytes. This finding supports those of Cormane et al. (3) and Carapeto et al. (2) that patients with AD can have increased numbers of IgE-positive lymphocytes in their blood. However, most patients in this study had less than 3% IgE-bearing lymphocytes and McGeady et al. (12) have reported normal numbers of such cells in AD. These varying results can depend upon technical factors such as different anti-

sera, but the selection of patients may also have an influence upon the results. The results of the present investigation indicate that the number of IgE-bearing lymphocytes may be correlated to the activity of the dermatitis, but the number of patients tested is, however, not sufficiently large to allow for wider conclusions to be drawn in this respect.

REFERENCES

1. Andersen, E. & Hjorth, N.: B lymphocytes, T lymphocytes and phytohaemagglutinin responsiveness in atopic dermatitis. *Acta Dermatovener (Stockholm)* 55: 345, 1975.
2. Carapeto, F. J., Stobo, J. D., Winkelmann, R. K. & Jordon, R. E.: T and B lymphocytes in chronic dermatitis. *J Invest Dermatol* 64: 297, 1975.
3. Cormane, R. H., Husz, S. & Hamerlinck, F.: Immunoglobulin- and complement-bearing lymphocytes in allergic contact dermatitis and atopic dermatitis. *Br J Dermatol* 90: 597, 1974.
4. Grove, D. I., Reid, J. G. & Forbes, I. J.: Humoral and cellular immunity in atopic eczema. *Br J Dermatol* 92: 611, 1975.
5. Hanifin, J. M.: Immunologic aspects of atopic dermatitis. *Cutis* 13: 479, 1974.
6. Hovmark, A.: An in vivo and in vitro study of cell-mediated immunity in atopic dermatitis. *Acta Dermatovener (Stockholm)* 55: 181, 1975.
7. Johansson, S. G. & Juhlin, L.: Immunoglobulin E in "healed" atopic dermatitis and after treatment with corticosteroids and azathioprine. *Br J Dermatol* 82: 10, 1970.
8. Jondal, M., Holm, G. & Wigzell, H.: Surface markers on human B and T lymphocytes. *J Exp Med* 136: 207, 1972.
9. Leguit, P., Meinesz, A., Zeijlemaker, W. P., Schellekens, P. T. A. & Eijssvoegel, V. P.: Immunological studies in burn patients. *Int Arch Allergy* 44: 101, 1973.
10. 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.
11. Luckasen, J. R., Sabad, A., Goltz, R. W. & Kersey, J. H.: T and B lymphocytes in atopic eczema. *Arch Dermatol* 110: 375, 1974.
12. McGeady, S. J. & Buckley, R. H.: Depression of cell-mediated immunity in atopic eczema. *J Allerg Clin Immunol* 56: 393, 1975.
13. Rajka, G.: Delayed dermal and epidermal reactivity in atopic dermatitis. *Acta Dermatovener (Stockholm)* 48: 186, 1968.
14. Schöpf, E.: Störung zellvermittelter Immunoreaktionen bei Neurodermitis atopica. *Dermatologica* 149: 210, 1974.
15. — Zellvermittelte Immunität bei Neurodermitis atopica. *Hautarzt* 25: 420, 1974.
16. Tada, T., Okumura, K. & Taniguchi, M.: Regulation of homocytotropic antibody formation in the rat. *J Immunol* 111: 952, 1973.
17. Thestrup-Pedersen, K., Ellegaard, J., Thulin, H. & Zachariae, H.: Mitogen and PPD responsiveness in severe atopic dermatitis. *J Invest Dermatol* 64: 296, 1975.
18. Wüthrich, B.: Zur Immunpathologie der Neurodermitis constitutionalis. Verlag Hans Huber, Bern, 1975.
19. Öhman, S. & Johansson, S. G. O.: Immunoglobulins in atopic dermatitis. *Acta Dermatovener (Stockholm)* 54: 193, 1974.

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