

AN IN VIVO AND IN VITRO STUDY OF CELL-MEDIATED IMMUNITY IN ATOPIC DERMATITIS

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Abstract. Cell-mediated immunity was studied in patients with atopic dermatitis. Tuberculin skin tests were performed on 70 patients and on 18 controls. The patients with "severe dermatitis" and those with high IgE levels were hyporeactive. Lymphocyte transformation tests were carried out in 33 patients and 21 controls. This showed that the patients were hyporeactive to PPD and herpes simplex antigen and this was most prominent in the patients having high IgE values. However, there was no clear, statistically significant difference between patients and controls with regard to their in vitro response to PHA.

Key words: Dermatitis, atopic; Hypersensitivity, delayed; Lymphocyte transformation; Tuberculin test; IgE

Considerable controversy still exists about the etiology and mechanisms of atopic dermatitis. Several authors (7, 13, 15, 16) have reported a tendency to non-reactivity to tuberculin skin tests in patients with atopic dermatitis and it has been discussed whether patients with atopic dermatitis have a low degree of delayed response to bacterial and viral antigens (10, 15, 16, 19). There have been reports of increased (4, 6), normal (2), and decreased (8, 13, 17) incidence of allergic contact dermatitis among patients with atopic dermatitis.

In vitro studies of the cellular immune system in patients with atopic dermatitis have been performed in a few cases. Fjelde et al. (5) reported a tendency to fewer mitoses in lymphocyte cultures stimulated with PHA among 14 patients with atopic dermatitis, as compared with 12 controls, but they could not draw any statistical conclusions since the material was too small and there were individuals in both the patient- and the control group who did not react at all to PHA. Lobitz et al. (10) found depressed in vitro cell-mediated immunity in 2 patients with active atopic dermatitis and both patients exhibited low responses to PHA in lymphocyte transformation tests.

Many (though not all) patients with atopic

dermatitis have a high serum level of IgE and there is a correlation between high IgE values and extensive dermatitis (1, 18, 21). There is strong evidence that allergic asthma and allergic rhinitis are IgE mediated. However, the role of reaginic antibodies in atopic dermatitis is not clear and dermatitis does not appear to be primarily caused by the presence of reagins (14). Patients with atopic dermatitis also seem to have increased levels of IgG and IgM (21) and it has been discussed whether this is caused by a high incidence of infections in these patients or whether it can be explained by other mechanisms. Parish et al. (14) suggested that persons with atopic diseases have a basic defect in the T-lymphocyte system. The T-lymphocytes might be activated by an endogenous stimulant, probably non-antigenic in nature, and this could give the dermatitis. Such a stimulation might exhaust the T-lymphocytes and could explain a low activity in the cell-mediated immune system. The failure of a hypothetical regulatory or inhibitory function of the T-cell system could also explain the abnormal immunoglobulin levels in patients with atopic diseases.

The aim of this investigation was to study whether lymphocyte transformation tests support the hypothesis of hyporeactive, cell-mediated immunity in patients with atopic dermatitis or whether they support the concept that abnormalities in the skin or other mechanisms can give "false negative" skin tests for delayed hypersensitivity. The investigation was performed with PPD tuberculin skin tests and lymphocyte transformation tests with PPD, herpes simplex antigen, and PHA. Since many patients with atopic dermatitis have high IgE levels and since there seems to be a correlation between the severity of the dermatitis and the IgE level, the findings were also correlated to the serum levels of IgE in the patients and to the extent of the dermatitis.

Table I. Severity of the dermatitis with regard to serum IgE levels in patients with atopic dermatitis

	Number	"Severe dermatitis"	
		Number	Per cent
IgE > 1 000 U/ml	30	20	67
IgE < 1 000 U/ml	38	5	13

MATERIALS AND METHODS

Patients were obtained from the Dermatologic Department of Södersjukhuset, Stockholm. Tuberculin skin tests were done on 70 patients with atopic dermatitis and 33 patients also participated in the lymphocyte transformation tests. The 33 patients who were selected for the lymphocyte transformation tests all suffered from active atopic dermatitis at the times when the tests were performed. They consisted of 16 women and 17 men and their ages varied between 17 and 52 years, with a mean of 28. Among the other patients, who only participated in the skin tests, there were also those who had recently suffered from dermatitis but had no active dermatitis when the tests were performed.

The patients were divided into two groups according to the extent of their skin involvement. "Severe dermatitis" = active dermatitis afflicting more than 1/3 of the body-surface. "Mild dermatitis" = active dermatitis on less than 1/3 of the body-surface, or inactive dermatitis. Tests were made on 68 of the patients to determine serum IgE levels (RIST, commercial method from Pharmacia). According to Oppé et al. (12) the normal range of IgE in non-allergic adults is 83-650 U/ml. In this investigation, IgE levels of more than 1 000 U/ml = "high IgE levels".

None of the patients had been on parenteral steroid medication for the last 6 months before the tests were performed.

The control group consisted of 4 patients with psoriasis and 17 healthy volunteers, 10 females and 11 males, in the age-range 18-43 years (mean, 32 years).

Skin tests were carried out on patients and controls by intracutaneous injections of 0.1 ml PPD-tuberculin 2 T.U. (Statens seruminstitut, Copenhagen, Denmark) in the forearm. The diameter of induration was measured after 72 hours and an induration of less than 6 mm was regarded as negative. In Sweden, BCG vaccination is obligatory during the first postnatal days of life. Those patients and controls who knew that they had been re-vaccinated with BCG during the last few years were not included in the *in vitro* tests with PPD.

Tissue culture technique

Before the tuberculin skin test was carried out, 20-40 ml venous blood was drawn under aseptic conditions and heparinised. The lymphocytes were separated by the Ficoll Isopaque method and washed three times in saline buffer. 10^6 lymphocytes were cultivated in a one ml volume of Eagle's suspension medium supplemented with 10% heat-inactivated human AB-serum and antibiotics. The tubes were gassed with 10% CO_2 and incubated at 37°. Cultures in triplicate were set up with the following mitogen, antigens, and controls:

Antigens and mitogen:

1. Purified protein derivate (PPD, Statens seruminstitut, Copenhagen) was used at a dose of $1 \mu\text{g}/10^6$ cells/ml.
2. Herpes simplex antigen and control preparation without herpes antigen (Microbiologiska centrallaboratoriet, Stockholm, Sweden) diluted 1/10, which was the lowest concentration giving good stimulation. Herpes antigen has been used earlier for *in vitro* tests to study cell-mediated immunity (9, 20).
3. Phytohaemagglutinin (Purified PHA, Burroughs-Wellcome, USA) was used as a mitogen for human T lymphocytes at a dose of $10 \mu\text{g}/10^6$ cells/ml.
4. Control cultures without antigen or mitogen.

Twenty-four hours before harvest, $2 \mu\text{Ci}$ in 0.1 ml saline of ^3H -thymidine was added to each tube. The cultures with PHA were harvested after 3 days in culture and the others after 5 days. At harvest, the tubes were poured into Sartorius Membrane filters in a multiple sample collector and the cells were washed with 0.9% saline. The filters were dried and 10 ml of a toluene scintillation fluid was added to each scintillation vial and thereafter the samples were counted in a liquid scintillation spectrometer. Data are presented as mean values of each triplicate. Ratios = counts/minute in stimulated cultures/counts/minute in control cultures.

Statistics

χ^2 -test and *t* test were used as statistical methods. To make it possible to perform statistical analysis of the logarithmically transformed values, low values were awarded to tubes with no stimulation at all, in order to overcome the problem with $\log 0_{10}$.

RESULTS

IgE levels

The IgE levels of 68 patients varied from less than 10 to 16 070 U/ml (mean: 1 650 U/ml and median 630 U/ml). 30/68 (44%) patients had IgE values of more than 1 000 U/ml.

Severity of the dermatitis

25/70 (36%) patients had "severe dermatitis". In the group with "high IgE levels" 20/30 (67%) had "severe dermatitis" as compared with 5/38 (13%) in the group with IgE less than 1 000 U/ml (Table I). This difference was statistically significant ($p < 0.01$). Table IV shows that the median value for IgE was highest in the group with "severe dermatitis". A high percentage of the patients selected for *in vitro* tests had "severe dermatitis" and this explains the high median value for IgE in this group.

Skin reactivity to PPD

The skin reactivity to PPD was negative in 29 of the 70 patients (39%) (Table II). A higher proportion

Table II. Results of tuberculin skin tests in patients with atopic dermatitis and controls

	Number	Tuberculin skin test negative	
		Number	Per cent
Total patients	70	29	39
"Severe dermatitis"	25	16	64
"Mild dermatitis"	45	13	29
IgE > 1 000 U/ml	30	17	57
IgE < 1 000 U/ml	38	12	32
Controls	18	3	17

was skin test negative in the group having "severe dermatitis" (16/25 = 64%) as compared both with the group with "mild dermatitis" (13/45 = 29%) ($p < 0.01$) and the controls (3/18 = 17%) ($p < 0.01$).

In the patient group exhibiting "high IgE levels", 17/30 (57%) were negative in their skin tests as compared with 12/38 (32%) in the group with IgE less than 1 000 U/ml. The difference between patients with "high IgE levels" and the controls, was statistically significant at the 0.02 level. In Table IV this is reflected in a high median value for IgE in the patient group with negative skin tests to PPD.

PPD in vitro

The results of thymidine uptake into blood lymphocytes after stimulation with PPD are documented in Fig. 1 and Table III. In the control group there was a correlation (corr. coefficient = 0.78) between in vivo and in vitro tests to PPD. Those who had the strongest reactions in vivo also gave the highest lymphocyte stimulations in vitro and the controls who had negative in vivo tests showed the lowest responses in vitro.

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

	Number	Mean value		Median value	
		Counts	Ratios	Counts	Ratios
Total patients	32	13 200	10.4	8 800	7.4
IgE > 1 000 U/ml	19	7 800	7.2	6 400	4.5
IgE < 1 000 U/ml	13	21 000	15.3	19 000	9.0
PPD skin test negative	19	6 700	6.1	2 800	3.7
PPD skin test positive	13	22 600	16.8	24 000	9.2
Controls	20	34 400	20.3	16 100	9.3

Table IV. Median values of serum IgE in patients with atopic dermatitis

	Number	IgE (U/ml)
Total	68	630
"Severe dermatitis"	25	2 050
"Mild dermatitis"	43	220
PPD skin test negative	29	1 750
PPD skin test positive	39	410
In vitro tested	33	1 750

Most of the patients being PPD-negative in vivo also responded weakly in vitro. However, some skin-negative patients got better responses in their in vitro tests than some skin-positive individuals. Statistical analysis of the logarithmically transformed values of the counts and ratios in Fig. 1 showed lower mean values for stimulation with PPD in the patient group than the controls (counts: $p < 0.01$, and ratios: $p < 0.05$). The patient group with "high IgE levels" had the lowest mean value for stimulation with PPD and was separated both from the controls (counts: $p < 0.001$, and ratios: $p < 0.01$) and from the patient group with IgE less than 1 000 U/ml (counts: $p < 0.05$, and ratios: $p < 0.05$).

A statistical analysis was also performed on the logarithmically transformed values of the counts and ratios in Fig. 2. This showed a lower mean value in the group with "severe dermatitis" than the mean value of the controls (counts: $p < 0.01$, and ratios: $p < 0.05$).

Herpes simplex in vitro

There was no significant stimulation or toxic effects from the control preparation without herpes simplex antigen. The result after stimulation with herpes simplex antigen in vitro was similar to that observed with PPD in vitro. Thus there were low degrees of stimulation in the group who had "high IgE levels". An analysis of the $\log_{10}$ values of the counts and ratios in Fig. 3 was carried out and this showed lower mean values in the patient group exhibiting "high IgE levels" than the mean values of the controls (counts: $p < 0.01$, and ratios: $p < 0.05$). The patient group as a whole had a lower mean value of the counts than the controls ($p < 0.02$). Three patients gave no response at all to herpes simplex antigen and 5 patients did not respond to PPD in vitro. Only one patient failed to respond to both herpes simplex antigen and PPD.

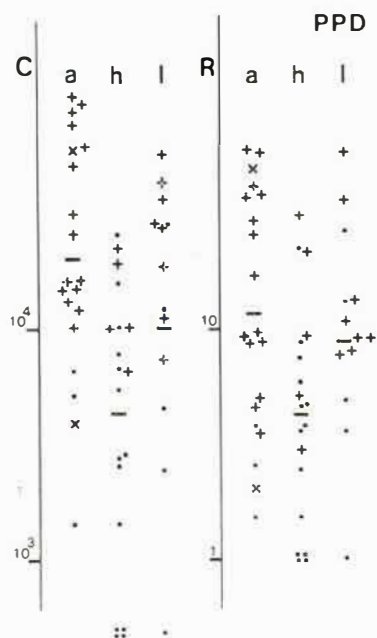


Fig. 1. Results of lymphocyte transformation tests with PPD in relation to IgE tabulated as $\log_{10}$ c.p.m. (C) and $\log_{10}$ ratios (R). a = controls, h = patients with "high IgE levels", l = patients having IgE less than 1 000 U/ml. + = PPD skin test positive, - = PPD skin test negative, x = PPD skin test not performed, — = mean value.

PHA in vitro

In the PHA response (Fig. 4) there was a tendency to hyporeactivity in the group with "high IgE levels", but the differences were not statistically significant. The 5 patients with "severe dermatitis", who did not respond at all to PPD in vitro (Fig. 2) had higher mean values for stimulation with PHA (counts: 168 000 c.p.m. and ratios: 75) as compared with the mean values of the controls (counts: 118 000 c.p.m. and ratios: 66) but neither of these differences were statistically significant.

DISCUSSION

This study confirms earlier investigations that many, but not all, patients with atopic dermatitis have a high serum level of IgE. Among the patients who had "high IgE levels" there was a larger proportion with "severe dermatitis" than among the patients with IgE values less than 1 000 U/ml. Other investigators (1, 18, 21) have also found that there is a correlation between the extent of the dermatitis and the IgE level.

The patients who had "severe dermatitis" and the patients with "high IgE levels" were more often PPD-negative in vivo than the controls, and this is in agreement with earlier observations (7, 13, 15, 16) showing that weak tuberculin skin reactions occur in patients having atopic dermatitis. PPD was used in vitro at a dose of $1 \mu\text{g}/10^6$ cells/ml and it has been shown that lymphocyte transformation test with PPD, in this low dose, when harvested early, correlates well to tuberculin skin test (11). In this investigation there was a good correlation between in vivo and in vitro reactivity to PPD. However, some skin-negative individuals gave stronger in vitro responses than some who were skin-positive. The explanation for this "overlapping" might be that it is difficult to standardise the laboratory conditions, when cultures are grown on different days. The investigation showed statistically significant hyporeactivity to PPD among the patients, most prominent among the patients who had "high IgE levels" and the results were similar, whether statistical analyses were made of the counts or of the ratios. However, the significances were sometimes lower when calculated on the ratios, as compared to calculations with the

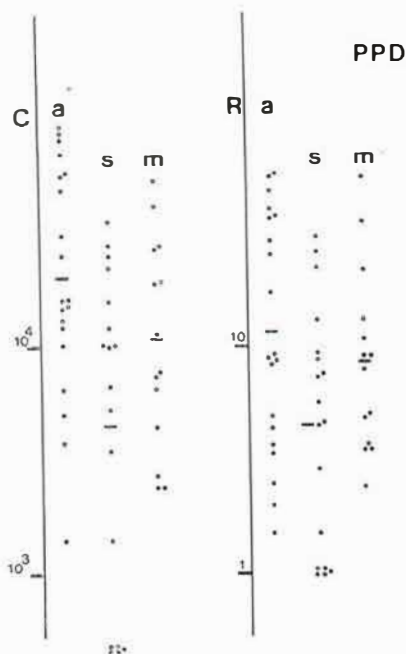


Fig. 2. Results of lymphocyte transformation tests with PPD in relation to severity of dermatitis tabulated as $\log_{10}$ c.p.m. (C) and $\log_{10}$ ratios (R). a = controls, s = patients with "severe dermatitis", m = patients with "mild dermatitis", — = mean value.

counts. This can be explained by the variations in the counts in the reference-tubes, which make the results more spread, when they are presented as ratios. The results of this investigation indicate that there is a hyporeactivity to PPD, not only in the skin tests of many atopics, but also in the in vitro tests and thus the hyporeactivity to PPD in patients with atopic dermatitis may have an immunologic basis. Since there was a good correlation between "severe dermatitis" and "high IgE levels" (Table I), it was no surprise to find relatively pronounced hyporeactivity to PPD in vivo and in vitro in the group having "severe dermatitis", as was the case with the patients who had high IgE values, but it is not possible from this investigation to draw any conclusions as to whether the hyporeactivity to PPD among patients with atopic dermatitis is best correlated to high IgE values or to the extent of the dermatitis.

The cell-mediated immune system is probably involved in the defence mechanism against herpes simplex. A high percentage of the population come into contact with this virus and become immunized. Serological evidence of herpes simplex infection has been found in up to 90% of adults (3). The

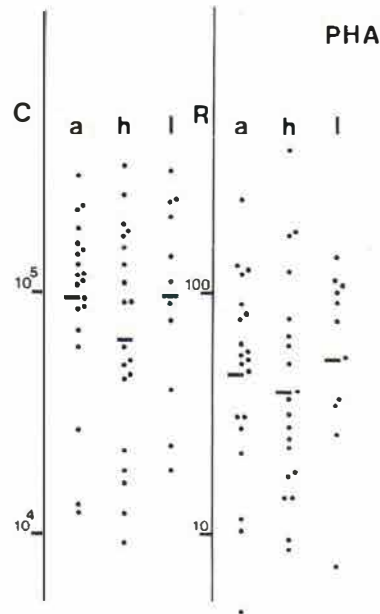


Fig. 4. Results of lymphocyte transformation tests with PHA in relation to IgE tabulated as $\log_{10}$ c.p.m.(C) and $\log_{10}$ ratios(R). a=controls, h=patients with "high IgE levels", l=patients having IgE less than 1 000 U/ml, — = mean value.

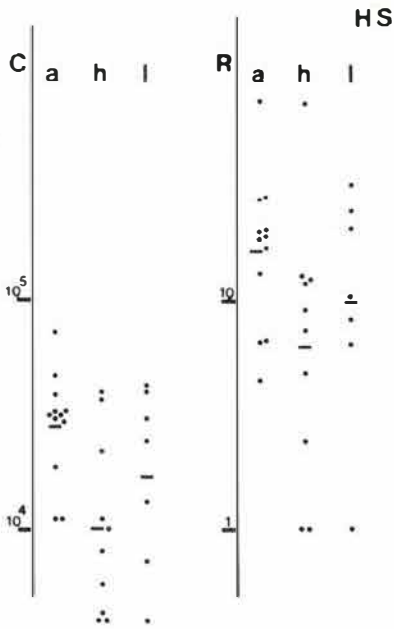


Fig. 3. Results of lymphocyte transformation tests with herpes simplex antigen in relation to IgE tabulated as $\log_{10}$ c.p.m.(C) and $\log_{10}$ ratios(R). a=controls, h=patients with "high IgE levels", l=patients having IgE less than 1 000 U/ml, — = mean value.

intention in this investigation was to use herpes simplex as a T-cell antigen and stimulation was expected in a high proportion of the controls, but since all the controls were stimulated (Fig. 3), it cannot be definitely stated whether this represents an antigenic or a mitogenic stimulation. However, initial tests which gave stronger stimulation when harvested on days 4–5 as compared with harvest on days 2–3 indicate that the responses were antigenic in nature. The results of the in vitro tests with herpes simplex antigen suggest a low cell-mediated response among patients who have atopic dermatitis. This might indicate a defective immunologic defence mechanism against herpes simplex virus, which perhaps can explain the severe complications of herpes simplex infections, which sometimes occur in patients with atopic dermatitis.

Lobitz et al. (10) have reported low in vitro response from PHA stimulation in 2 patients with active atopic dermatitis but it is not possible to draw any wide conclusions from 2 patients. The tendency to hyporeactivity to PHA among the patients in this investigation was not statistically significant. Some of the patients with the most severe dermatitis and high IgE values were among

those who had the strongest responses to PHA, although they had no reactions to PPD. Thus it seems as if patients with severe atopic dermatitis can have normal activity in their immune system if stimulated with "T-cell mitogen", but usually have low responses if the T-lymphocytes are stimulated with "T-cell antigens". The explanation for this might be that the abnormality in the T-lymphocyte system of the patients with atopic dermatitis is caused more by a dysfunction of the T-cells than by a reduction in their number.

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Received October 7, 1974

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