Clin. exp. Immunol. (1977) 27, 118–126.

PPD and mitogen responsiveness of lymphocytes from patients with atopic dermatitis

K. THESTRUP-PEDERSEN, J. ELLEGAARD, H. THULIN & H. ZACHARIAE *Institute of Medical Microbiology, Department of Internal Medicine, Aarhus County Hospital, and Department of Dermatology, Marselisborg Hospital, University of Aarhus, Aarhus, Denmark*

(*Received* 16 *July* 1976)

SUMMARY

Lymphocytes from twenty-five patients with atopic dermatitis were investigated for their *in vitro* reactivity to stimulation with tuberculin (PPD), lipopolysaccharide (LPS), phytohaemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM). The response to a low dose of Con A was increased, and the reactivity in unstimulated cultures tended to be lower than similar cultures from the control group. Addition of inactivated autologous plasma to the cultures had an inhibitory effect, when the plasma came from patients with high levels of IgE. The patients' *in vitro* reactivity to PPD in a leucocyte migration test was equal to that found in normal persons and no effect was observed after addition of autologous serum. The mean percentage of E rosette forming cells was significantly reduced in patients with high levels of IgE. The number of EAC rosette forming cells was within normal range. It is hypothesized that the observations could reflect the existence of suppressor mechanisms in patients where the immune system is strongly stimulated.

INTRODUCTION

It is a common clinical observation that patients with severe atopic dermatitis have decreased resistance to a variety of infections. Cell-mediated immune reactions, which are expressed by thymus derived (T) lymphocytes, have been noted to be deficient in some patients with atopic dermatitis (Palacios, Fuller & Blaylock, 1966; Jones, Lewis & McMarlin, 1973; Lobitz, Honeyman & Winkler, 1972) and other studies have presented evidence for a decreased number of T-lymphocytes in patients with this disease. However, lymphocytes from atopic patients showed a normal reactivity *in vitro* to stimulation with phytohaemagglutinin, which mainly stimulates T lymphocytes, except when the cells were cultured in autologous plasma (Luckasen *et al.*, 1974; Schöph & Böhringer, 1974; Andersen & Hjorth, 1975).

Recent reports on the function of lymphocytes from patients with atopic dermatitis have measured only a few *in vitro* parameters of T lymphocyte functions. We have used the most common *in vitro* techniques for measuring cell-mediated immune reactions to measure the reactivity of lymphocytes from patients with atopic dermatitis and compared the results with observations on a group of healthy persons.

PATIENTS AND METHODS

Twenty-five patients were studied. They were divided into two groups according to their level of IgE in serum. Group I consisted of fourteen patients with high levels of IgE ranging from 1290 u up to 9100 u (normal range 30–500 u, 1 u corresponds to approximately 2 μg/ml). Their age ranged between 12 and 64 years with a median age of 22 years, Group II contained eleven patients with normal or low levels of IgE. Their age ranged between 13 and 48 years with a median age of 22 years. Generally, the group consisting of patients with high IgE levels had the most severe and most widespread

Correspondence: Dr K. Thestrup-Pedersen, Institute of Medical Microbiology, University of Aarhus, DK 8000 Aarhus C, Denmark.

disease. These patients were almost all studied immediately after being referred to the department and were in an active stage of their disease. Several had widespread secondary infections mainly with staphylococci. Some patients in Group II had also a very active disease, but none displayed severe secondary infections on their skin. All patients had suffered from atopic dermatitis since early childhood and had received treatment with potent local steroids from time to time, but not at the time of the investigation. Beside antihistamines none had received systemic treatment. A control group of fourteen persons without any skin disease was included, their age ranged between 13 and 64 years with a median age of 25 years.

The level of IgE in serum was measured with a radioimmunosorbent test (Johansson, Bennich & Wide, 1968) and was performed at Medicinsk Laboratorium, Copenhagen.

Lymphocyte transformation test, LTT. 20 ml of venous blood was drawn into a test tube containing 400 i.u. of phenolfree heparin and separated on Isopaque-Ficoll (Böyum, 1974). This separation procedure allows plasma to be withdrawn together with the mononuclear cells. The cells were washed twice in TC-199 with 5% A serum and heparin 20 i.u./ml. A white cell differential count was obtained and the lymphocyte concentration was adjusted to 1×10^6 per ml; 2×10^5 lymphocytes were cultured in 0·2 ml TC-199 in microculture plates (Linbro Chemical Co., IS-MRC-96, New Haven, CT) with the addition of either 15% A serum or 5% A serum plus 10% autologous plasma. The A serum, hereafter called standard serum, was a pool from four normal blood-donors (A, Rhesus pos.) and was used due to difficulty in obtaining sufficient amounts of AB serum. Both serum and plasma was inactivated at 56°C for 30 min before use.

The lymphocytes were stimulated with purified protein derivative of tuberculin (PPD) in a concentration of 10 μg per ml (Statens Seruminstitut, Copenhagen), lipopolysaccharide (LPS) 100 μg/ml (Difco, 3923-25 Bacto) and pokeweed mitogen (PWM) 5 μl/ml (Grand Island Biological Company). These concentrations were found to give the highest incorporation of thymidine in normal lymphocytes in the culture system used. Cultures were terminated after 5 days with these antigen/mitogens. Lymphocytes were also stimulated with phytohaemagglutinin (PHA) in concentrations of 0·5 μg/ml, 2·5 μg/ml and 5·0 μg/ml (Wellcome MR 68, PHA-P), concanavalin A (Con A) 10 μg/ml and 25 μg/ml and PWM 5 μl/ml and 25 μl/ml. The largest concentration of each mitogen had earlier been found to give the optimal response in the presently used culture technique; the lower dose gave a suboptimal response. The duration of these cultures was 3 days.

Twenty-four hours before termination of the cultures 0·02 μCi of 2-^{14}C thymidine was added (Amersham, England, specific activity > 56 mCi/mM), and the cells were harvested on a glass fibre filter using a Skatron cell harvester. The amount of labelled thymidine incorporated was counted in a Tri-Carb scintillation counter. The results are expressed as disintegrations per minute (dpm) $\times 10^{-3}$ after correction for quenching and efficiency.

The results from the LTT are evaluated in two ways. Firstly, the *in vitro* reactivity of the lymphocytes to antigens and mitogens, when cultured in 15% standard serum, is compared among both groups of patients to the reactivity of the lymphocytes from the control group. Such a comparison would reveal differences in the reactivity of the lymphocytes themselves. Secondly, the influence of autologous plasma on *in vitro* reactivity of the lymphocytes can be seen in cultures where 10% autologous plasma plus 5% standard serum was used instead of 15% standard serum. The reason for adding standard serum to the cultures together with autologous plasma is the observation by Mangi, Dwyer & Kantor (1974) that the response of lymphocytes to mitogen stimulation shows a large variation when plasma from only one person is used. This variation can be significantly reduced when a pool of plasma is added also. Again the results from both groups of patients are compared to the results in the control group. A third comparison was made between the results obtained in cultures from one person differing only in the type of serum used, i.e. either 15% standard serum or 5% standard serum plus 10% autologous plasma.

The statistical evaluations were made using a Wilcoxon test for two samples except in the last comparison where a Wilcoxon test for pair differences has been applied. A P value below 0·05 was considered significant.

Leucocyte migration test, LMT. The leucocyte migration test (LMT) was performed in accordance with the agarose technique (Clausen, 1973) and is detailed elsewhere (Thulin, Thestrup-Pedersen & Ellegaard, 1975). Venous blood was collected in 10 ml polystyrole tubes containing 250 i.u. heparin. After sedimentation at 37°C for 1 hr the leucocyte-rich plasma was transferred to thrombotest tubes and spun at 220 g for 5 min. The pellets were washed three times in Hanks's balanced salt solution. Half of the leucocytes were resuspended in TC-199 with 10% inactivated AB serum, the other half in TC-199 with 10% inactivated autologous serum. PPD was used as antigen in a concentration of 250 μg/ml and added to half of the cells in AB serum and half of the cells in autologous serum. The remaining cells without added PPD were used as control. After incubation of all cells at 37°C for 30 min, 7 μl of leucocyte suspension was placed in each well in the agarose plate. Six antigen-containing wells and six control wells were set up with leucocytes in AB serum and in an identical way with leucocytes in autologous serum. This gave a total number of twenty-four wells for each patient. All plates were incubated at 37°C and the migration was measured after 24 hr by means of a projection microscope. Migration indices (MI) were calculated as the ratio between the average areas of antigen-containing wells and the control wells. Two MIs were obtained for each person, one for leucocytes in AB serum and one for leucocytes in autologous serum.

Counting T and B lymphocytes. Cells from venous blood were prepared as described above (Böyum, 1974) and depleted of monocytes and other phagocytic cells by incubating the washed leucocytes with carbonyl iron, 40 mg/ml, for 30 min at room temperature under slow rotation. The iron particles and phagocytic cells were then removed with a magnet. This procedure reduces the number of monocytes to approximately 5%. The number of T lymphocytes was estimated by counting lymphocytes which formed rosettes with sheep red blood cells (E rosettes) (Jondal, Holm & Wigzell, 1972); bone marrow derived (B) lymphocytes were measured by counting lymphocytes forming rosettes with sheep red cells coated with anti-sheep antibody, IgM, plus complement (EAC rosettes) (Aiuti *et al.*, 1974).

RESULTS

Results in the LTT with 15% *standard serum* (statistical significant differences are marked with ▲ in tables and figures)

Lymphocytes from atopic patients were found to react normally *in vitro* to PPD, LPS, PWM (5-day culture) and to three concentrations of PHA (Tables 1 and 2 and Fig. 1). The background synthesis of DNA in unstimulated cultures from patients in Group I (high levels of IgE) was less than that found with cells from the control group (Tables 1 and 2) although not statistically significant ($P<0{\cdot}10$). The lowest dose of Con A induced a significantly greater response in cultures from patients in Group I compared to the response in the control group and a similar tendency was found for patients in Group II ($P<0{\cdot}10$, Fig. 2). However, this difference in reactivity was not found with a larger dose of Con A (Table 3). Patients in Group II (low levels of IgE) had a reduced response to both concentrations of PWM (Fig. 3 and Table 3; for PWM 25 μl/ml: $P<0{\cdot}10$) and in unstimulated cultures of 3 days duration (Table 2, $P<0{\cdot}05$).

Results in the LTT with autologous plasma; comparison between patient groups and control group (statistical significant differences are marked with * in tables and figures)

Patients in Group I had a significantly reduced response of their lymphocytes when stimulated with PPD and PWM (5-day cultures) and in unstimulated 5-day cultures (Table 1). Autologous plasma seems thus to have reduced the *in vitro* reactivity of the lymphocytes. Fig. 1 and Table 2 shows that autologous plasma did diminish the reactivity in cultures from patients in Group I stimulated with two of three

TABLE 1. Mean results in a lymphocyte transformation test (LTT) after stimulation with various antigen/mitogens in optimal concentrations and in unstimulated cultures (5-day culture). The results are given as disintegrations per minute (d/min) × 10^{-3}. PPD: purified protein derivative of tuberculin, LPS: lipopolysaccharide, PWM: pokeweed mitogen. Standard serum: 15% pooled, inactivated A serum in cultures. Autologous plasma: 5% standard serum and 10% inactivated patients plasma in cultures

	Group I: patients with high IgE			Group II: patients with low IgE			Control group		
Mitogen	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n
PPD, 10 μg/ml									
Standard serum	2·7	±3·07	9	2·5	±1·66	8	3·6	±2·42	7
Autologous plasma	*1·6	±1·96 ●	9	*1·9	±1·16	8	4·4	±3·08	6
LPS, 100 μg/ml									
Standard serum	0·7	±0·71	9	1·5	±1·15	7	0·7	±0·82	7
Autologous plasma	0·5	±0·70	9	0·9	±0·87	7	0·6	±0·50	6
PWM, 5 μl/ml									
Standard serum	6·7	±3·53	9	7·6	±2·87	8	9·2	±2·73	7
Autologous plasma	*4·5	±3·15	9	6·1	±3·14	8	8·6	±4·10	6
Unstimulated cultures (5-day culture)									
Standard serum	0·114	±0·061	9	0·289	±0·081	8	0·309	±0·208	7
Autologous plasma	*0·137	±0·122	9	0·481	±0·393	8	0·640	±0·531	6

The upper horizontal line in Tables 1–3 is the result from cultures with 15% standard serum. Each group of patients has been compared with the control group, and signs for significant differences—if any—are placed on the left side of the figures (▲: $P<0{\cdot}05$). Similarly, the lower line is the result from cultures with added autologous plasma. Each group of patients has been compared with the control group and signs for significant differences are placed on the left side of the figures (*: $P<0{\cdot}05$). The statistical evaluation has been performed with Wilcoxon's test for two samples.

A comparison has been made between the results obtained when lymphocytes from the same person were stimulated in either standard serum or autologous plasma. Signs for significant differences (●: $P<0{\cdot}05$) are placed on the right side of the figures in the lower line. A Wilcoxon test for pair differences has been used for statistical evaluation. $\bar{x}$: mean, s.d.: standard deviation, n: number of patients.

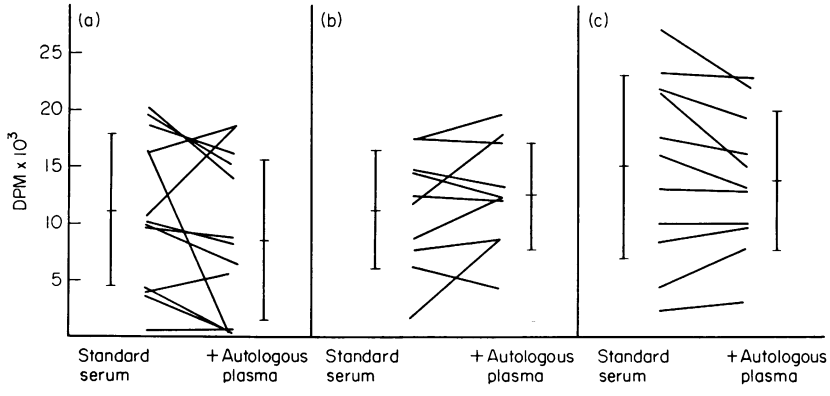

FIG. 1. Individual results in an LTT after stimulation with phytohaemagglutinin in a suboptimal concentration, 2·5 μg/ml. The lines connect the pair of results obtained from one person when culturing the lymphocytes in standard serum or autologous plasma. DPM × 10^{-3}: disintegrations per minute. The vertical lines indicate mean ± 1 s.d. For further explanation, see legend to Table 1. (a) High levels of IgE; (b) low levels of IgE; (c) normal.

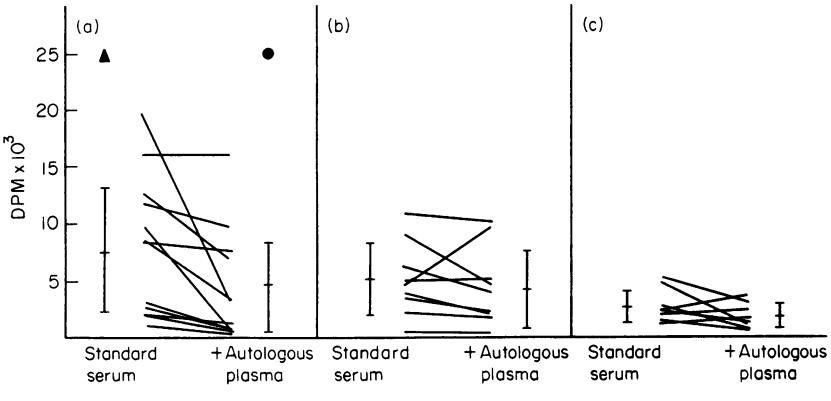

FIG. 2. Individual results in an LTT after stimulation with concanavalin A in a suboptimal concentration, 10 μg/ml. For further explanation, see legend to Table 1 and Fig. 1. (a) High levels of IgE; (b) low levels of IgE; (c) normal.

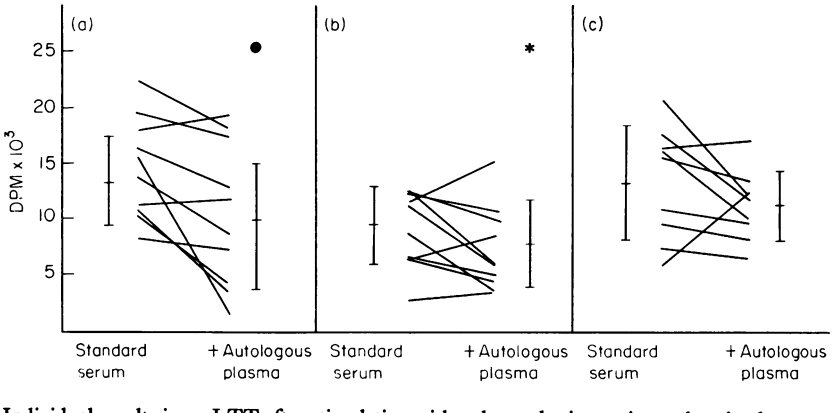

FIG. 3. Individual results in an LTT after stimulation with pokeweed mitogen in a suboptimal concentration, 5 μl/ml. For further explanation, see legend to Table 1 and Fig. 1. (a) High levels of IgE; (b) low levels of IgE; (c) normal.

doses of PHA compared with the control group although the reduction is not statistically significant ($P<0{\cdot}10$ in both comparisons). Fig. 2 shows that the addition of plasma diminished the response to the low dose of Con A so much that the lymphocyte reactivity was no longer significantly greater than the response in the control group ($P<0{\cdot}10$). PWM stimulation (3-day culture) gave a response within the normal range (Fig. 3 and Table 3).

The response of lymphocytes from patients in Group II did not differ from normal subjects when the cells were cultures in autologous plasma, except after stimulation with PPD (Table 1) and PWM, 5 μl/ml (Fig. 3). The decrease in reactivity of unstimulated 3-day cultures (Table 2) was seen too when the cultures contained standard serum alone. A continued increased response at the low dose of Con A ($P<0{\cdot}10$) was still found (Fig. 2).

TABLE 2. Mean results in an LTT after stimulation with phytohaemagglutinin (PHA) in two concentrations and in unstimulated cultures (3-day culture). For further explanation, see legend to Table 1

	Group I: patients with high IgE			Group II: patients with low IgE			Control group		
Mitogen	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n
PHA, 0·5 μg/ml									
Standard serum	3·6	±5·30	12	1·4	±2·06	9	1·0	±0·93	8
Autologous plasma	2·1	±2·60	12	3·0	±2·88	9	2·3	±4·24	8
PHA, 5·0 μg/ml									
Standard serum	13·1	±5·17	13	14·6	±4·38	10	15·4	±4·73	9
Autologous plasma	10·3	±7·30	12	14·0	±4·57	10	14·9	±4·69	9
Control cultures (3-day culture)									
Standard serum	0·121	±0·077	13	▲0·079	±0·034	10	0·332	±0·403	10
Autologous plasma	0·157	±0·286	12	*0·069	±0·028	10	0·145	±0·087	11

TABLE 3. Mean results in an LTT after stimulation with concanavalin A (Con A) and pokeweed mitogen (PWM) (3-day culture) in optimal concentrations. For further explanation, see legend to Table 1

	Group I: patients with high IgE			Group II: patients with low IgE			Control group		
Mitogen	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n
Con A, 25 μg/ml									
Standard serum	9·5	±6·12	12	7·4	±4·18	10	6·3	±3·89	11
Autologous plasma	3·5	±2·72 ●	12	6·5	±3·95	10	5·5	±5·76	11
PWM, 25 μl/ml									
Standard serum	13·0	±3·83	9	10·5	±3·60	9	14·3	±4·58	11
Autologous plasma	8·6	±5·69 ●	9	10·2	±4·18	9	12·3	±3·57	11

Comparison of stimulation of cells from one subject cultured in either standard serum or autologous plasma (statistical significant differences are marked with ● in tables and figures)

Reactivity of lymphocytes from one person was compared when cultured in either standard serum or autologous plasma. In the cultures from normal subjects no significant changes were found in reactivity in any of the twelve different culture conditions. For some antigen/mitogens the reactivity was higher, when the lymphocytes were cultured in 10% autologous plasma and 5% standard serum and in other situations this was reversed.

Cultures from patients in Group II were also found not to display different reactivity with either standard serum or with autologous plasma even after stimulation with PPD and PWM (3-day cultures). In Group I, however, the reactivity of stimulated cultures was always less after the addition of autologous plasma. The decrease was significant after PPD stimulation (Table 1) and after Con A and PWM stimulation at both concentrations used (Table 3 and Figs. 2 and 3). The reactivity to PWM (5-day cultures, Table 1) and to PHA, 2·5 and 5·0 μg/ml, was diminished by autologous plasma (Fig. 1 and Table 2; $P<0{\cdot}10$ in all three comparisons). Nine of thirteen patients demonstrated an impaired response in their cultures with autologous plasma when a dose of 2·5 μg/ml of PHA was used (Fig. 1).

In six of twelve patients in Group I the *in vitro* reactivity that occurred in autologous plasma to 10 μg/ml of Con A was half the reactivity found when pooled serum was used (Fig. 2). At 25 μg/ml of Con A this number increased to eight of twelve patients. In two patients autologous plasma completely inhibited the *in vitro* reactivity of the cells to Con A. These two plasma samples could also inhibit normal lymphocytes from several healthy subjects to respond to mitogens. The inhibitory activity could be eliminated by dialysing the plasma for 48 hr against sterile, isotonic saline in a cellulose dialysing tube which permitted molecules with a molecular weight below 22,000 to enter the dialysate.

When passing one of the plasma samples through a Sephadex G-200 column the fraction containing the inhibitory activity appeared in the last peak of low molecular weight substances, but not in the three first peaks containing immunoglobulin. At the time of occurrence of the inhibitory activity, the two patients had a deterioration in their disease including widespread secondary infections with staphylococci. Plasma was taken from one of these patients 2 months later, when he was clinically improved. No inhibitory activity was then observed.

The inhibitory effect of plasma was not correlated to the level of IgE in the patients. However, inactivation of serum or plasma destroys IgE and to assess the influence of high amounts of IgE on the *in vitro* reactivity of lymphocytes, eight experiments were performed in which cultures from atopic patients received IgE containing homologous plasma, which was either not heat-inactivated or inactivated for various periods of time. No significant change was observed between the cultures with complete IgE and heat-destructed IgE.

Leucocyte migration test

The results from the LMT appear in Table 4. The mean results of the individual MI values are given and show a tendency of a decrease in the release of migration inhibition factor in patients in Group I compared with the patients in Group II and the control group. However, no significant differences were found. The use of autologous, inactivated serum instead of AB serum did not influence the results.

T and B lymphocytes

Table 5 shows that the number of E rosette forming cells was significantly lower in Group I than in the control group. A slight, but insignificant increase was seen for EAC rosette forming cells. The same pattern was observed for patients in Group II, but the difference was not significant. No significant correlation was found between the number of E rosette forming cells and the level of IgE in serum.

TABLE 4. Mean results of migration indices (MI) after addition of PPD to lymphocytes in a leucocyte migration test (LMT). AB serum: 10% inactivated pooled AB serum in cultures. Autologous serum: 10% inactivated patients serum in cultures

	Group I: patients with high IgE		Group II: patients with low IgE		Control group	
	AB serum	Autologous serum	AB serum	Autologous serum	AB serum	Autologous serum
MI	0·94	0·93	0·90	0·89	0·89	0·88
SD	0·09	0·05	0·05	0·08	0·08	0·08
n	14	14	10	10	14	14

TABLE 5. Mean percentages of E rosette forming cells (T lymphocytes) and EAC rosette forming cells (B lymphocytes) in patients and controls. ■: $P<0.01$

	Group I: patients with high IgE		Group II: patients with low IgE		Control group	
	E	EAC	E	EAC	E	EAC
$\bar{x}$	40·1%■	26·9%	46·9%	23·0%	53·6%	22·1%
SD	9·23	9·68	10·62	2·93	7·11	7·17
n	14	14	8	8	14	14

DISCUSSION

Considerable evidence has accumulated to suggest that cell-mediated immunity is impaired in patients with atopic dermatitis. It has been reported (Palacios *et al.*, 1966) that only 76% of patients with atopic dermatitis were sensitized by dinitroclorobenzene (DNCB) compared to more than 90% of normal subjects. The ability to reject allogeneic skin grafts was, however, normal and so was the antibody responses to various antigens. In people with clinical history of previous reactivity to *Rhus oleoresin* from poison ivy, patch tests with this extract induced a positive response in 61% of otherwise healthy subjects, but only in 15% of patients with atopic dermatitis (Jones *et al.*, 1973). Skin anergy, lack of sensitization with DNCB, depleted paracortical (T cell) areas in lymph nodes and decreased *in vitro* reactivity to stimulation with PHA was found in two patients with severe atopic dermatitis (Lobitz *et al.*, 1972). The depressed PHA response became normal after a clinical improvement in the patients.

The present results seem to indicate that lymphocytes from atopic patients, cultured in homologous serum, respond normally to several different mitogens (PPD, LPS, PHA, PWM 5-day culture). However, findings of altered reactivity to a low dose of Con A together with a diminished DNA synthesis in the unstimulated cultures may reflect an intrinsic disturbance of lymphocyte functions or a change in the occurrence of functionally different subpopulations of T and B lymphocytes. Our reason for using a suboptimal concentration of mitogen is the observation by Fitzgerald (1971) and more recently by Malavé, Layrisse & Layrisse (1975) that a lymphocyte deficiency could be seen as a decreased *in vitro* response at low, suboptimal concentrations of PHA whereas an increased dose of PHA leads to a normal response of the lymphocytes. Thus, the increase in the reactivity to the low dose of Con A would not have been discovered, if only the optimal concentration had been used.

Further evidence for changed immunological reactivity in some patients with atopic dermatitis was found when autologous plasma was added to the lymphocyte cultures. Autologous plasma did not change the reactivity of normal lymphocytes and it is often more supportive for growth of lymphocytes than homologous plasma (Mangi *et al.*, 1974). It is our experience and that of others (Dwyer, personal communication) that there is no difference in the *in vitro* reactivity of cultures with serum or plasma. Lymphocytes from patients with low levels of IgE (Group II) did not show significant decreases in their *in vitro* reactivity after addition of autologous plasma. However, when autologous plasma was added to lymphocytes from patients with high levels of IgE all stimulated cultures showed a diminished reactivity and in many cases the decrease was significant. The plasma factor(s) which is responsible for the observed suppression does not seem to be IgE although it was found in plasma from patients with high levels of IgE. It has been reported that histamine in certain concentrations is able to suppress the *in vitro* reactivity of normal lymphocytes when stimulated with PHA (Jones & Artis, 1975). However, it seems unlikely to be the factor concerned. Although patients with atopic dermatitis have a slightly elevated skin histamine (Johnson *et al.*, 1960), there is no indication that these patients have larger amounts of circulating free histamine in their plasma. Free histamine is normally cleared from the blood stream within a few minutes (Beall & van Arsdel, 1960).

Lymphocytes from patients in both groups released migration inhibition factor normally even in the

presence of autologous serum. The significant decrease in the percentage of E rosette forming cells is in accordance with previous findings (Luckasen *et al.*, 1974; Andersen & Hjorth, 1975) and could support the hypothesis that production of IgE antibodies is influenced by T lymphocytes. Several investigations indicate an inverse relationship between the number of T lymphocytes in blood and the IgE level in serum (Rosenbaum & Dwyer, personal communication).

It is likely that the present findings are due to the strong stimulation of the patients' immune system. This could lead to the triggering of active suppressor mechanisms in an attempt to diminish the involvement of larger numbers of lymphocytes (Kantor, 1975). A hypothesis which could account for our findings would need the following premises. Existence of suppressor lymphocytes in human (Sampson, Grotelueschen & Kauffman, 1975), immunosuppressive factors being markers for suppressor lymphocytes (Kantor, 1975), an inverse relationship between number of T lymphocytes and IgE and that PHA mainly stimulates a subpopulation of T helper (?) lymphocytes, suboptimal doses of Con A mainly stimulates suppressor lymphocytes and PWM also stimulates B cells. Evidence for the stimulation of different subpopulations by PHA and Con A is now reported (Jacobsson & Blomgren, 1974; Rawson & Huang, 1975) and it appears that a subpopulation of T cells stimulated by Con A exerts immunosuppressive functions (Tardieu & Daguillard, 1975).

An explanation of our findings could therefore be as follows. Patients with severe atopic dermatitis (Group I) had the strongest stimulation of their immune system leading to pronounced function of suppressor lymphocytes. This was seen in a high reactivity to a suboptimal concentration of Con A, in immunosuppressive factors in plasma and a low number of T helper (?) lymphocytes. This decrease in T helper (?) lymphocytes was reflected in a low response to a suboptimal concentration of PHA (2·5 μg/ml, Fig. 1). Patients in Group II were less severely affected by their disease, but although they had a normal number of T lymphocytes, normal or low levels of IgE and no clear evidence for immunosuppressive factors in plasma, they still may show signs of a suppressor function through the increased reactivity of their lymphocytes to the suboptimal concentration of Con A and the low reactivity in the unstimulated 3-day cultures and in PWM stimulated cultures (3-day culture). The higher reactivity to PWM stimulation found in Group I compared with Group II may be explained by the fact that at least some B lymphocytes were active in producing IgE and therefore may be more sensitive to stimulation with PWM as measured after 3 days of culture.

Although these considerations are hypothetical the presented data suggests that regulatory mechanisms are expressed in patients with severe atopic dermatitis and it makes further investigations of these patients desirable.

We wish to thank Mrs Karen Skibsted, Mrs Vibeke Thulin, Mrs Karen M. Hansen and Miss Kate Sommer for skilful technical assistance. Mr Walther Sørensen solved our problem of the transportation of samples in an excellent way. This work has received support from P. Carl Petersen Foundation and the NOVO Foundation.

REFERENCES

AIUTI, F., CEROTTINI, J., COOMBS, R., COOPER, M., DICKLER, H., FRØLAND, S., FUDENBERG, H., GREAVES, M., GREY, H., KUNKEL, H., NATVIG, J., PREUDHOMME, J., RABELLINO, E., RITTS, R., ROWE, D., SELIGMANN, M., SIEGAL, F., STJERNSWÄRD, J., TERRY, W. & WYBRAN, J. (1974) Identification, enumeration and isolation of B and T lymphocytes from human peripheral blood. *Scand. J. Immunol.* **3**, 521.

ANDERSEN, E. & HJORTH, N. (1975) B lymphocytes, T lymphocytes and phytohaemagglutinin responsiveness in atopic dermatitis. *Acta Derm.-Ven.* **55**, 345.

BEALL, G. & VAN ARSDEL, P. (1960) Histamine metabolism in human disease. *J. clin. Invest.* **39**, 676.

BÖYUM, A. (1974) Separation of blood leucocytes, granulocytes and lymphocytes. *Tissue Antigens*, **4**, 269.

CLAUSEN, J. (1973) Leucocyte migration technique: some technical details. *Acta allergol.* **28**, 351.

FITZGERALD, M.G. (1971) The establishment of a normal human population dose-response curve for lymphocyte cultures with PHA. *Clin. exp. Immunol.* **8**, 421.

JACOBSSON, H. & BLOMGREN, H. (1974) Responses of mouse thymic cells to mitogens. A comparison between phytohaemagglutinin and concanavalin A. *Cell. Immunol.* **11**, 427.

JOHANSSON, S., BENNICH, H. & WIDE, L. (1968) A new class of immunoglobulins in human serum. *Immunology*, **14**, 265.

JOHNSON, H., DE ORE, G., LASCHEID, W. & MITCHELL, F. (1960) Skin histamine levels in chronic atopic dermatitis. *J. invest. Derm.* **34**, 237.

JONDAL, M., HOLM, G. & WIGZELL, H. (1972) Surface markers on human T and B lymphocytes. *J. exp. Med.* **136**, 207.

JONES, H.E. & ARTIS, W.M. (1975) Histamine inhibition of human lymphocyte transformation. *Clin. Res.* **23**, 452A.

JONES, H.E., LEWIS, C.W. & MCMARLIN, S.L. (1973) Allergic contact sensitivity in atopic dermatitis. *Arch. Dermatol.* **107**, 217.

KANTOR, F.S. (1975) Infection, anergy and cell-mediated immunity. *New Engl. J. Med.* **292**, 629.

LOBITZ, W.C., HONEYMAN, J.F. & WINKLER, N.W. (1972) Suppressed cell-mediated immunity in two adults with atopic dermatitis. *Brit. J. Derm.* **86**, 317.

LUCKASEN, J.R., SABAD, A., GOLTZ, R.W. & KERSEY, J.H. (1974) T and B lymphocytes in atopic eczema. *Arch. Dermatol.* **110**, 375.

MALAVÉ, I., LAYRISSE, Z. & LAYRISSE, M. (1975) Dose-dependent hyporeactivity to phytohemagglutinin in systemic lupus erythematosus. *Cell. Immunol.* **15**, 231.

MANGI, R.J., DWYER, J.M. & KANTOR, F.S. (1974) The effect of plasma upon lymphocyte response *in vitro*. Demonstration of a humoral inhibitor in patients with sarcoidosis. *Clin. exp. Immunol.* **18**, 519.

PALACIOUS, J., FULLER, E.W. & BLAYLOCK, W.K. (1966) Immunological capabilities of patients with atopic dermatitis. *J. invest. Derm.* **47**, 484.

RAWSON, A.J. & HUANG, T.C. (1975) The response to phytohemagglutinin or to concanavalin A as a probe for subpopulations of human peripheral blood lymphocytes. *Cell. Immunol.* **17**, 310.

SAMPSON, D., GROTELUESCHEN, C. & KAUFFMAN, H.M. (1975) The human splenic suppressor cell. *Transplantation*, **20**, 362.

SCHÖPH, E. & BÖHRINGER, D. (1974) Cellular immunity in atopic dermatitis. *Der Hautarzt*, **25**, 420.

TARDIEU, M. & DAGUILLARD, F. (1975) Fractionated T lymphocytes can inhibit mitogenic responses through dialyzable factors. *Cell. Immunol.* **19**, 148.

THULIN, H., THESTRUP-PEDERSEN, K. & ELLEGAARD, J. (1975) *In vitro* parameters of cell-mediated immune reactions in healthy individuals following immune-stimulation attempts with levamisole. *Acta allergol.* **30**, 9.