

## An *in vitro* study of lymphocytes in patients with atopic dermatitis

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(Received 1 December 1981; revision accepted for publication 25 April 1982)

### Summary

The authors studied the T- and B-lymphocyte sub-populations, the stimulation of the lymphocytes with phytohaemagglutinin and pokeweed mitogen, the non-specific T suppressor activity and the amounts of serum IgE in a group of thirty-four patients with atopic dermatitis. Comparison with a group of 100 healthy controls revealed a diminution in the T sub-population, an increase in the B sub-population, no change in the response index to stimulation with phytohaemagglutinin or pokeweed mitogen, a diminished T-suppressor activity and an increase in the level of serum IgE.

### Introduction

Data from *in vitro* immunological studies on patients with atopic dermatitis have been numerous and controversial. Thus, for example, an increase in the IgE concentrations of these patients, first described by Juhlin *et al.* (1969) and confirmed by others (Bousquet *et al.*, 1978; Jones, Inoye & McGerit, 1975; Hanifin & Gottlieb, 1974; O'Loughlin *et al.*, 1977), led to its inclusion among the criteria for the diagnosis of atopic dermatitis. Some authors (Rimbaud *et al.*, 1973; Ohman & Johansson, 1974), however, found normal amounts of IgE and others found IgE against a variety of antigens (Rajka, 1975; Hoffman, 1975). There appears to be no correlation between change in the IgE level and the severity of the disease (Stone, Gleich & Muller, 1976).

The results of the studies of lymphocyte response in subjects with atopic dermatitis stimulated with different mitogens are more variable. Thus, although Bousquet *et al.* (1978) and Gottlieb and Hanifin (1974) find no alteration, Lobitz, Honeyman and Winkler (1972) and Andersen and Hjorth (1975) describe a fall in the response after stimulation with phytohaemagglutinin when the culture is prepared in the presence of autologous serum.

The study of lymphoid populations, in the majority of patients, show a decrease in numbers of the T population (Hanifin & Gottlieb, 1974; Carapeto, Winkelmann & Jordan, 1976; Luckasen *et al.*, 1974) with variable results in the numerical determina-

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tion of the B-lymphoid sub-population (Bousquet *et al.*, 1978). The discovery in the majority of cases of atopic dermatitis of a high level of IgE and an alteration in the T-lymphoid sub-population (Rogge & Hanifin, 1976) may indicate an alteration in the balance between T suppressor/T helpers with a diminution in the activity of the former. As a result a hyperproduction of IgE (Strannegård & Strannegård, 1979) occurs. In man, the study of Schuster *et al.* (1979) in patients with atopic dermatitis and with a high IgE level, does not show a diminished T suppressor activity in relation to the control group. This led these authors to suggest that the non-specific activation of the suppressor cells with concanavalin-A (Rich & Pierce, 1973) provides a population that is different from that which regulates the production of IgE.

All these data, some of which are rather controversial, prompted us to study the B- and T-lymphoid sub-populations (T-E<sub>i</sub> and T-E<sub>a</sub>), the stimulation of lymphocytes with phytohaemagglutinin (in the presence of both autologous and foetal calf serum) and pokeweed mitogen, the non-specific T-suppressor activity induced by concanavalin-A and the level of serum IgE in a group of thirty-four patients with atopic dermatitis.

## Materials and methods

### *Patients*

We studied thirty-four patients (males and females, adults and children) with atopic dermatitis. The patients were selected on the basis of a family history of atopic dermatitis, prurigo, lichenification and characteristic localization of eczema. We found symptoms of allergic respiratory disease in the majority of the patients who were subjected to skin tests to detect immediate hypersensitivity to a battery of pneumo-allergens.

### *Controls*

The control group consisted of 100 normal and healthy subjects (males and females, adults and children) free of personal and family immunologic processes of whatever origin: allergic respiratory disease, atopic dermatitis, medication or food allergy, parasitosis, autoimmune diseases, etc. The controls were subjected to the same tests as the study group.

### *Isolation of lymphoid suspension*

From each patient or control we obtained, by sterile venous puncture, 30 ml blood. The serum was separated from 5 ml and the remaining 25 ml was defibrinated, diluted 1:1 with phosphate-buffered saline (PBS) and deposited on a gradient of Ficoll-Metrizoate (density: 1078). After centrifugation for 20 min at 600 *g* the cell layers were collected and washed three times with PBS. After studying the viability they were resuspended in Rosewell Park Memorial Institute solution (RPMI 1640) and adjusted to  $1 \times 10^6$  and  $4 \times 10^6$  cells/ml.

### *Numerical distribution of B- and T-lymphoid populations*

To count the number of T-E<sub>i</sub> lymphocytes (for designations T-E<sub>i</sub> and T-E<sub>a</sub>, see Fundenberg, Wybran & Robbins, 1975) aliquots of a suspension of lymphocytes ( $4 \times 10^6$  cells/ml) and sheep red blood cells (SRBC) in PBS at 1% (approx.  $200 \times 10^6$

cells/ml) were mixed and incubated for 5 min at 37°C. After centrifugation for 5 min at 200 *g* they were left for the night at 4°C. The readings (by two technicians) was carried out on a hematocytometer and 200 cells were counted. Lymphocytes with three or more attached red cells were considered to be positive.

To count the T-E<sub>a</sub> population, 0.1 ml lymphocyte suspension ( $4 \times 10^6$  cells/ml) and 0.1 ml foetal calf serum (FCS) (inactivated), which had been absorbed with SRBC, were mixed and incubated for 60 min at 37°C. After adding 0.1 ml SRBC the suspension was centrifuged at 200 *g* for 5 min, before being gently resuspended. The readings were carried out as in the case of the T-E<sub>i</sub> lymphocytes. For the study of the B-lymphoid population 0.1 ml lymphocyte suspension ( $4 \times 10^6$  cells/ml) and 0.1 ml antiserum anti-Ig fragment F(ab)<sub>2</sub> labelled with fluorescein (Behring) were mixed, incubated for 45 min at 4°C and then washed three times with PBS. The cellular sediment was then deposited on a slide after which a reading of the cells was taken with a fluorescent Leitz microscope (200 fluorescent and non-fluorescent cells were counted by two technicians).

*Stimulation with phytohaemagglutinin (PHA) and pokeweed mitogen (PWM)*

Aliquots of lymphocyte suspensions (0.1 ml) adjusted to  $1 \times 10^6$  cells/ml were distributed in different wells of a flat-bottomed microtest plate (Nunc, Denmark). We added 0.1 ml of a solution of PHA-M (Gibco) in culture medium RPMI 1640 at a concentration of 10 µg/ml or PWM (Gibco) at a concentration of 10 µg/ml to some of the wells. All the wells contained 0.1 ml of RPMI 1640 with 20% of FCS or RPMI with 20% of autologous serum (AS). The plates were then incubated at 37°C in a humid atmosphere of 5% CO<sub>2</sub> for 92 hr. Then 18 hr before the completion of the culture 0.2 µCi of thymidine <sup>3</sup>H was added to each well. The cells were collected on a fibre glass filter using the Skatron AS cell collector and were then transferred to a vial with 5 ml of scintillation liquid which was read on a Beckman counter 7000.

The results were recorded as counts per minute (ct/min) and the response index (RI) was expressed by dividing the means obtained in the presence of PHA or PWM and the control wells.

*Study of non-specific T-suppressor activity induced by concanavalin-A (con-A)*

Two millilitres of lymphocyte suspension ( $1 \times 10^6$  cells/ml) with 30 µg con-A/ $1 \times 10^6$  cells (suppressor cells) and 2 ml only in culture medium (control cells) were incubated for 24 hr at 37°C in a atmosphere of 5% CO<sub>2</sub>. The remaining cells were distributed in wells on a microtest plate (Nunc, Denmark) at a concentration of  $1 \times 10^5$  cells/well (responder cells). After a period of 24 hr the suppressor cells and the control cells were washed and incubated with mitomycin-C (100 µg/tube) for 30 min at 37°C. They were then washed three times, resuspended in the original volume using RPMI 1640 with 20% serum. We added  $1 \times 10^5$  suppressor cells to some of the responder cells and  $1 \times 10^5$  control cells (concentration for each well) to the rest of the responder cells, and later we added PHA-M (Gibco) at a concentration of 10 µg/ $1 \times 10^6$  cells to half of the wells with responder cells. The plates were then incubated at 37°C in a humid atmosphere of 5% CO<sub>2</sub> for 72 hr. We added 0.2 µCi thymidine <sup>3</sup>H to each well 18 hr before completion of the culture and the procedure followed was the same as described for the stimulation with mitogens.

The non-specific T-suppressor activity was expressed as a percentage by this formula:

$$1 - \frac{(\text{Responder cells} + \text{suppressor cells with PHA}) - (\text{Responder cells} + \text{suppressor cells without PHA})}{(\text{Responder cells} + \text{control cells with PHA}) - (\text{Responder cells} + \text{control cells without PHA})} \times 100$$

### Levels of IgE

IgE concentrations were measured using the method of radioimmunoassay Phadebas Prist (Pharmacia).

### Results

Figure 1 shows the mean values and deviations of the T-E<sub>t</sub>-lymphoid sub-population of the control group ( $60 \pm 8.3$ ) and of the study group ( $49.8 \pm 14.3$ ) ( $P < 0.001$ ). The same figure also shows the mean values and deviations of the T-E<sub>a</sub> sub-population of

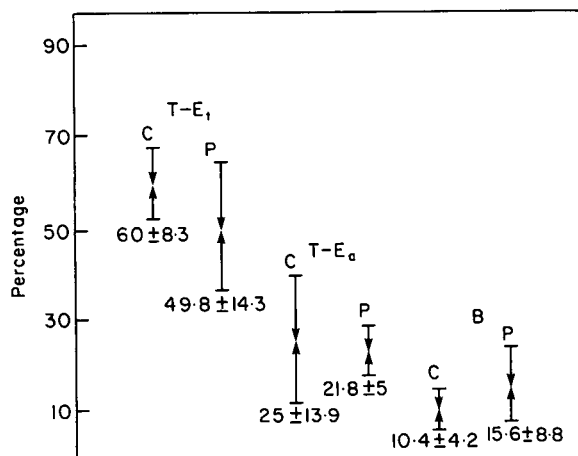


Fig. 1. Lymphoid subpopulations T-E<sub>t</sub>, T-E<sub>a</sub> and B in study group with atopic dermatitis and in control group. C, controls; P, patients.

the control group ( $25 \pm 13.9$ ) and of the study group ( $21.8 \pm 5$ ) ( $P > 0.05$ ). Finally Fig. 1 shows the mean values and deviations of the B lymphocytes of the control group ( $10.4 \pm 4.2$ ) and of the patient group ( $15.6 \pm 8.8$ ) ( $P < 0.001$ ).

Figure 2 shows the RI's of the control group stimulated with PHA in the presence of FCS ( $73 \pm 31$ ) and PHA in AS ( $71 \pm 34$ ) and with PWM in the presence of FCS ( $68 \pm 26$ ) and also the RI's of the study group ( $86 \pm 52.5$ ), ( $77.4 \pm 57.7$ ) and ( $71.6 \pm 56.4$ ) respectively ( $P > 0.05$ ).

Figure 3 shows the means values and deviations of the non-specific T-suppressor activity induced by con-A of the control group ( $34 \pm 13.9$ ) and of the study group ( $2.6 \pm 19.5$ ) ( $P < 0.001$ ).

Finally, Fig. 4 shows the mean values and deviations of the IgE level of the control group ( $67 \pm 53$  u/ml) and of the study group ( $369 \pm 109.5$  u/ml) ( $P < 0.001$ ).

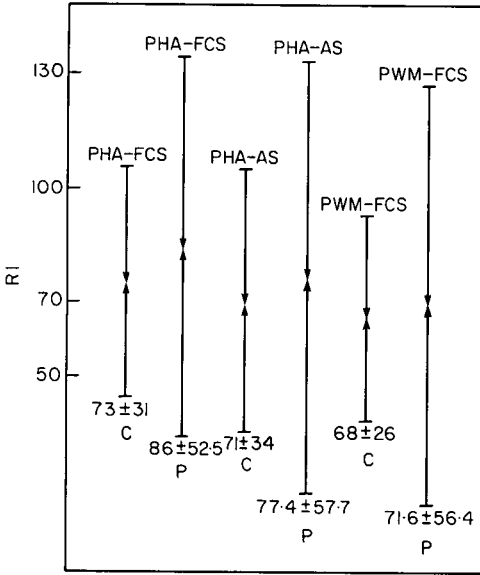


Fig. 2. Response index to PHA and PWM in study group and control group. C, controls; P, patients; RI, response index; FCS, foetal calf serum; AS, autologous serum.

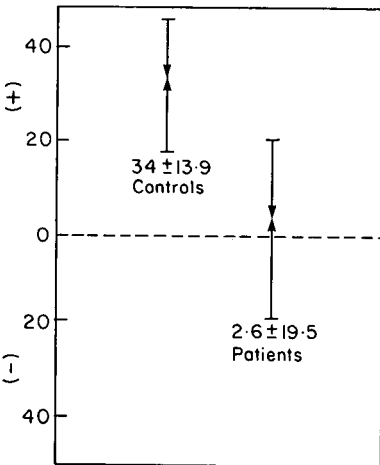


Fig. 3. T suppressor activity induced by con-A in control group and study group.

## Discussion

The susceptibility of patients with atopic dermatitis to vaccine, herpes simplex, warts and molds is well known. This indicates an altered cellular immunity in these patients (Hanifin & Lobitz, 1977). Though the susceptibility could also be related to intrinsic abnormalities in the skin of these patients, the existence of decreased delayed cellular responses to various antigens including those derived from molds and virus (Hovmark, 1975; McGeady & Buckley, 1975) tends to support the hypothesis of an alteration in

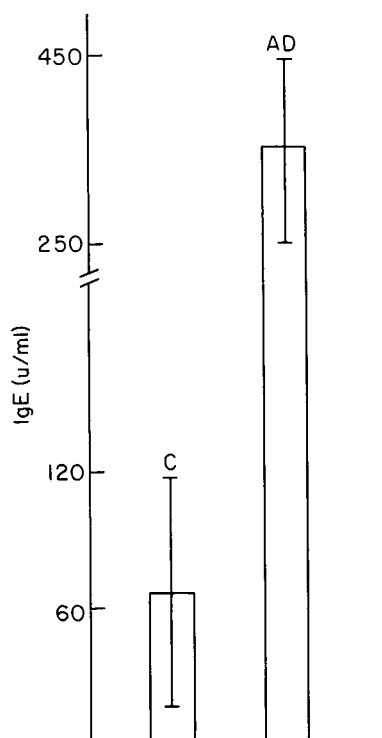


Fig. 4. IgE levels in control group and study group. C, controls; AD, atopic dermatitis.

the immune response. The data concerning the alteration in lymphoid populations seems to indicate, in general, a decrease in numbers of the T-E<sub>i</sub> population according to the study carried out by Bousquet *et al.* (1978). Differences in number of T-E<sub>i</sub> did not correspond to decreases in numbers of T-E<sub>a</sub> cells. This may be due to techniques used. Strannegård and Strannegård (1978) when comparing the results of various authors, stressed the need to consider the fact that the T deficiency in atopy is only detected when the methods used permit the enumeration of the T cells with low avidity for SRBC (T-E<sub>i</sub>). These findings are consistent with the results obtained from our present study (T-E<sub>i</sub>:  $P < 0.001$ ; T-E<sub>a</sub>:  $P > 0.05$ ).

The levels of the B sub-population are more variable since, although McGeady and Buckley (1975) and Bousquet *et al.* (1978) among others find them high which is consistent with our findings, Strannegård, Lindholm and Strannegård (1976) and Grove, Reid and Forbes (1975) do not. On the other hand, Cormane, Hamerlinck and Husz (1975) find that between 14 and 31% of the lymphocytes of patients with atopic dermatitis reveal positive fluorescence for IgE and Lobitz *et al.* (1972) found, in a lymph node biopsy of a patient with atopic dermatitis, a depletion of the T-paracortical areas, an increase in the zones of B localization as well as the presence of IgE receptors in the B cells.

The response of the lymphocytes stimulated with PHA is, in our data, similar to that of the control group using both FCS ( $P > 0.05$ ) or AS ( $P > 0.05$ ) respectively. These findings are consistent with those of Bousquet *et al.* (1978), Hovmark (1977) and Thestrup-Pedersen *et al.* (1977). However, Lobitz *et al.* (1972) found a fall in the

response to this mitogen, and Andersen and Hjorth (1975), only detected the reduced response when preparing the culture in the presence of autologous serum. Rachelefsky *et al.* (1976) found a decreased response to PHA in subjects with atopic dermatitis when using weak concentrations of mitogen whereas the response was normal when using an optimal concentration of PHA. The possible discrepancy between the alteration of the T sub-population and the normal response to stimulation with PHA may be explained by the lineal non-function of the response of the T cells to PHA (Parker, 1980). Our results of the stimulation with PWM showed normal responses corresponding with those of Bousquet *et al.* (1978) and Strannegård *et al.* (1976).

The study of non-specific T-suppressor activity induced by con-A showed the existence of a low level of activation in patients with atopic dermatitis ( $2.6 \pm 19.5$ ) compared to the control group ( $34 \pm 13.9$ ;  $P < 0.001$ ). These data are not consistent with the findings of Schuster *et al.* (1979) who found values that were similar in both atopic dermatitis and the control group.

Our mean values of IgE levels in patients with atopic dermatitis were higher than those of the control group ( $369 \pm 109.5$ ), ( $67 \pm 53$ ) respectively ( $P < 0.001$ ) which is consistent with the majority of the results by other authors (Juhlin *et al.*, 1969; Bousquet *et al.*, 1978; Jones *et al.*, 1975; Hanifin & Gottlieb, 1974; Schuster *et al.*, 1979; Hanifin & Lobitz, 1977; McGeadey & Buckley, 1975). An alteration in the regulation mechanism of IgE taking into consideration the increase in the amount of IgE related to immune cellular depression whether artificial (Lakin, Grave & Sell, 1975) or congenital (Kikkawa *et al.*, 1973) could give rise to a hyperproduction, with the presence of IgE molecules of low affinity for a wide range of antigens that would form complexes and when being deposited in the skin would cause the dermatological lesions presented by patients with atopic dermatitis (Johns & Stranworth, 1977).

All the evidence taken together is thought to indicate a change in T sub-population which in weak suppressor activity could in turn result in increased formation of IgE and the subsequent clinical manifestations of the disease.

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