

## AN *IN VITRO* STUDY OF IgE PRODUCTION IN SEVERE ATOPIC DERMATITIS

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**Abstract.** Peripheral blood lymphocytes from 4 patients with severe atopic dermatitis and with high serum IgE levels produced measurable amounts of IgE *in vitro* in repeated tests. These patients had increased numbers of IgE-bearing peripheral blood lymphocytes on at least one test occasion. No measurable IgE production *in vitro* was found in 6 other patients with atopic dermatitis and in 3 healthy controls. Inhibition of the IgE production was observed following treatment with PHA, Con A, PWM, mixed lymphocyte culture and radiation. LPS and histamine induced neither definite stimulation nor inhibition of IgE production. Supernatants from Con A stimulated cells were used in tests for suppressor factors. The hypothesis that depressed suppressor function of the T cells might be responsible for the tendency to increased IgE production in atopic dermatitis is discussed.

The pathogenesis of atopic dermatitis (AD) is still unclear. Immunological disturbances such as high serum IgE levels are often present (2, 6, 20). Hyporeactivity in the cell-mediated immunity has also been demonstrated in patients with AD when using both *in vivo* (6, 13) and *in vitro* (6, 9, 15) methods. Since patients with AD have signs of disturbed T cell function, hypofunction of the suppressor activity of T cells may explain the tendency to increased IgE production in atopic diseases.

In a previous study (7) 3 AD patients were described who had increased numbers of IgE-bearing peripheral blood lymphocytes during periods of exacerbation of severe dermatitis. The initial purpose of the present investigation was to study whether peripheral blood lymphocytes from these 3 patients could produce IgE *in vitro*, thus indicating that the IgE-bearing cells (or some of them) could be precursors of IgE-producing cells. As a result of the observation of IgE production *in vitro*, the study was expanded to include more patients. Various factors conceivably influencing IgE production, such as addition of mitogens, mixed lymphocyte culture (MLC) stimulation, histamine, radiation, and lymphokines, were also studied.

## MATERIAL AND METHODS

### *Patients and controls*

The patients were 10 adults with severe AD and high serum levels of IgE ( $>2000$  ng/ml). The healthy controls were matched with regard to age and sex.

The 4 patients with measurable IgE production *in vitro* were 2 males and 2 females aged 23, 31, 37 and 64 years. In all of them the AD had started in childhood and they had a tendency to long periods of generalized eczema or erythrodermic skin and high serum levels of IgE. They had experienced asthma and/or rhinitis but the dermatitis was the dominant atopic manifestation in all 4 cases. One of the patients received oral corticosteroid medication during the test period. Cells from these 4 patients were tested on several occasions for IgE production *in vitro*.

### *Cell culture technique*

The lymphocytes were separated by the Ficoll Isopaque method, as described previously (6). The cells were washed four times in saline buffer and  $2 \times 10^6$  cells/ml were cultivated in a 0.5 ml volume of Mishell-Dutton medium supplemented with antibiotics and 5% heat-inactivated calf serum. The tubes were gassed with 10% CO<sub>2</sub> and incubated at 37°C for 5 or 7 days. After shaking and centrifugation, IgE determinations were made on the supernatants (PRIST, Pharmacia, Uppsala, Sweden). One U=2 ng/ml. *In vitro* IgE production was assessed as IgE in the supernatant after the culture period minus IgE in the supernatant from tubes in which the cells had been killed by repeated freezing and thawing at the initiation of the culture period.

Cultures were set up using the following reagents:

1. Concanavalin A (Con A) 50 µg/ml, 10 µg/ml, 2 µg/ml, 0.1 µg/ml and 0.01 µg/ml (Pharmacia Fine Chemicals, Uppsala Sweden)
2. Phytohaemagglutinin (PHA) 10 µg/ml (Wellcome Ltd., Beckenham, Kent, England)
3. Pokeweed mitogen (PWM) 4 µg/ml (Serva Finbiochemica, Heidelberg, GFR)
4. Lipopolysaccharide (LPS) 100 µg/ml (obtained from Prof. T. Holme, Dept. of Bacteriology, Karolinska Institute, Stockholm, Sweden)
5. Histamine HCl  $10^{-3}$  and  $10^{-4}$  M (Vitrum AB Stockholm, Sweden)

Mixed lymphocyte cultures (MLC) were prepared by mixing  $10^6$  cells from one AD patient with  $10^6$  cells from another AD patient or a healthy control and cultured in 1

Table I. Results of IgE production in vitro and immunofluorescence studies of IgE-bearing cells in 4 patients with severe atopic dermatitis

| Pat. no. | IgE/serum (ng/ml) | IgE production in vitro (ng/ml) | IgE-bearing cells (%)                       |
|----------|-------------------|---------------------------------|---------------------------------------------|
| I        | 15 600            | 4.3–16.4; mean: 9.5 (3 tests)   | <3, <3, 6 (3 tests)                         |
| II       | 23 000            | 4.4–15.2; mean: 7.9 (6 tests)   | <3, <3, 9 (3 tests)                         |
| III      | 52 000            | 5.8–15.6; mean: 10.3 (4 tests)  | <3, 5, 7, 12, 3 (2 <sup>a</sup> ) (5 tests) |
| IV       | 120 000           | 9.8–54.0; mean: 34.2 (5 tests)  | 4 (2 <sup>a</sup> ) (1 test)                |

<sup>a</sup> Results after repeated washing of the cells at 37°C.

ml medium supplemented with 5% calf serum. The effects of radiation (50 R, 300 R, 3 000 R) were also studied.

Duplicate cultures were not run in all experiments, because of limitations of available cell numbers, but were done whenever the initial cell yield so permitted.

Since Con A could possibly bind to IgE (8), tests were performed with IgE-containing supernatants and patient sera, which were incubated at 37°C for 24 h with Con A 50 µg/ml. The Con A did not alter the IgE levels in the supernatants or in the patient sera. Thus Con A in this concentration did not influence the IgE determinations when using the PRIST method.

#### Test for suppressor factors

Two million lymphocytes in 1 ml medium with 5% calf serum were stimulated with Con A (50 µg/ml, 10 µg/ml and 2 µg/ml). Cultures without Con A were used as controls. After 24 h incubation the cells were washed 4–6 times with BSS in order to remove Con A and IgE in the supernatants. Subsequently the cells were reincubated in fresh medium with 5% calf serum and after a further 3 days the supernatants were harvested. Supernatants from one AD patient and one healthy control were tested for factors suppressing the IgE production in vitro in cells from another AD patient. The supernatants were used in a final dilution of 1:6. Percentage IgE production = (IgE production in cultures with supernatants minus IgE in supernatants × 1/6) × 100 / IgE production in cultures without supernatants.

#### Test for IgE-bearing cells

The number of IgE-bearing cells was determined by an immunofluorescence technique described previously (7). Only cells judged to be lymphocytes with positive membrane fluorescence were counted. In two tests the number of IgE-bearing cells was determined both after six repeated washing steps, both at room temperature and at 37°C before the incubation with the fluorescein-labelled antihuman IgE serum. The percentage of lymphocytes and basophils in peripheral blood was calculated by differential counting.

## RESULTS

Significant IgE production (>3 ng/ml) was found in cultures from the 4 patients with the highest serum levels of IgE. These patients were tested several

times (Table I). Small quantities of IgE were found in supernatants from cells which had been killed by repeated freezing and thawing before incubation (<1–4 ng/ml; mean value 1.4 ng/ml in 18 tests). The results were essentially the same whether the IgE determinations were performed immediately after the cells had been killed, or after 5–7 days. These values were subtracted from the IgE levels in supernatants from the viable cultured cells in order to determine IgE production. Significant IgE production was found on 18 occasions in 21 tests (4.3–54.0 ng/ml; mean value 16.0 ng/ml) (Table I). No measurable IgE production was found in cells from 6 other AD patients with high serum levels of IgE and 5 healthy controls.

The results of stimulation tests and radiation are shown in Table II. Stimulation with PHA, Con A, PWM, MLC and radiation resulted in inhibition of IgE production. Strong inhibition was obtained with Con A in concentrations of 50 µg/ml and 10 µg/ml but low concentration of Con A (10<sup>-2</sup> µg/ml) produced no inhibition. In controls with <sup>3</sup>H-thymidine

Table II. Results of IgE production in vitro after mitogen stimulation, MLC stimulation, and radiation in patients with severe atopic dermatitis

|                                   | No. of tests | Results (% of unstimulated cultures) |
|-----------------------------------|--------------|--------------------------------------|
| PHA, 10 µg/ml                     | 3            | 17, 27, 65                           |
| Con A, 50 µg/ml                   | 6            | 0, 22, 5, 28, 32, 17                 |
| PWM, 4 µg/ml                      | 3            | 42, 5, 59                            |
| LPS, 100 µg/ml                    | 4            | 137, 100, 105, 75                    |
| Histamine HCl, 10 <sup>-3</sup> M | 4            | 128, 61, 118, 88                     |
| Radiation                         |              |                                      |
| 50 R                              | 2            | 35, 45                               |
| 300 R                             | 2            | 21, 9                                |
| 3 000 R                           | 2            | 0, 2                                 |
| MLC                               |              |                                      |
| patient+control                   | 2            | 5, 10                                |
| patient+patient                   | 2            | 3, 12                                |

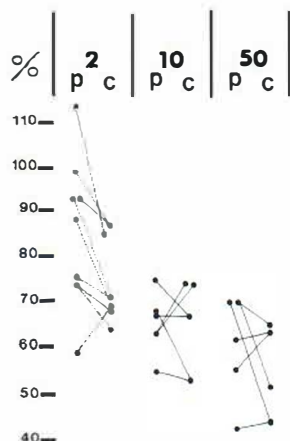


Fig. 1. Results of tests with supernatants from Con A stimulated cells expressed as % of IgE production in cultures without supernatants. 2=supernatant from cells stimulated with 2 µg/ml Con A. 10=supernatant from cells stimulated with 10 µg/ml Con A. 50=supernatant from cells stimulated with 50 µg/ml Con A. P=supernatant from AD patient, C=supernatant from healthy control. Results of different supernatants tested on cells from one patient on the same occasion are interconnected.

incorporation Con A 50 µg/ml and PHA 10 µg/ml gave optimal stimulation. LPS and histamine caused no certain stimulation or inhibition of the IgE production.

The results of the tests with supernatants from Con A stimulated cells are shown in Fig. 1. Supernatants from unstimulated cells induced no suppression. The supernatants from stimulated cells suppressed *in vitro* IgE production, but the suppression was not pronounced. When using supernatants from suboptimally stimulated cells (Con A=2 µg/ml) there was a tendency to diminished suppression from AD patients as compared with supernatants from healthy controls, and in 8 of 9 test pairs the supernatants from AD patients gave less inhibition than the supernatants from the controls.

The 4 patients with significant IgE production *in vitro* demonstrated >3% IgE-bearing (Ficoll-Isopaque separated) cells at least once in repeated tests (Table I). In two tests IgE-bearing cells were still seen after washing of the cells at 37°C. The other AD patients and the controls all demonstrated <2% IgE-bearing cells. None of the patients who had increased numbers of IgE-bearing cells were found to have more than 1% basophils in their peripheral blood leukocyte counts.

## DISCUSSION

Four patients with severe AD and very high serum IgE levels produced IgE *in vitro* in repeated tests. The results tally with those of Patterson et al. (11) who found that peripheral blood lymphocytes from two patients with very high IgE levels in serum produced IgE *in vitro*. However, in this study 6 other patients with AD and the controls did not produce measurable amounts of IgE *in vitro*. This indicates that with this method IgE production *in vitro* can be expected only in those patients with the most severe AD and highest serum IgE levels.

Increased percentages of IgE-bearing cells were found only in those patients whose cells could produce IgE *in vitro*. Increased numbers of IgE-bearing lymphocytes in patients with atopic diseases have been described by some authors (2, 3) whereas others have reported no such increase (9). Atopic patients may have IgE-bearing basophils (14). In this investigation, however, none of the patients were found to have more than 1% basophils in their peripheral blood leukocyte counts. It has been shown that immunoglobulins which are adsorbed to cells can be eluted by repeated washing at 37°C (12). This was tested in 2 patients. After washing at 37°C, IgE-bearing lymphocytic cells could still be shown, indicating that the IgE on the cell surfaces of these cells was not labile. The results indicate that at least some of the IgE-bearing cells found might be precursors of IgE-producing cells.

Signs of depressed cell-mediated immunity in AD have been found by several authors (6, 9, 13, 15) and correlation between a decrease in the T cell percentage and a rise in the concentration of serum IgE has also been reported (18). It has been shown in animal systems that T cells have a regulatory (helper and suppressor) function on the IgE production *in vivo* (17). IgE production is probably thymus dependent in man too (5). It has thus been speculated as to whether a depressed suppressor function of T cells might be responsible for the tendency to increased IgE production in humans with atopic diseases (2, 6, 7, 9, 15). The results of this investigation showed that agents that stimulate T cells (PHA, Con A, PWM) caused decreased IgE production, whereas a B cell mitogen such as LPS caused no significant decrease or increase in the IgE production. This supports the hypothesis that IgE production can be regulated (suppressed) by T cells also in man. The tendency to high IgE production in

atopics might thus be caused by a relative dysfunction of the suppressor activity of the T cells. The same may be true for other diseases with abnormal T cell function and a tendency to high serum IgE levels, e.g. mycosis fungoides and Wiskott-Aldrich syndrome.

Some authors have claimed that histamine (10) or radiation (17) may have specific inactivating effects on suppressor T cells in animal systems. Increased histamine sensitivity of T cells in atopic patients has also been reported (4, 16). However, in this test system, histamine gave no positive increase in the IgE production, and high levels of radiation probably damaged the IgE producing cells. B cell stimulation (LPS a B cell mitogen and PWM which stimulates both T and B cells) gave no definite increase of the IgE production in vitro. One explanation of this can be that in these patients the IgE-producing cells were already maximally stimulated in vivo and could not be further stimulated by B cell mitogens.

Waldman et al. (19) have described a "suppressor cell test" for human IgG, IgM and IgA production. A variation of this "MLC method" was used in order to test whether T cells from healthy controls (with healthy suppressor T cells) suppressed IgE production in vitro. In these "mixed cell cultures" the IgE production was greatly reduced. However, this was also the case when cells from 2 IgE producing patients were mixed. One probable explanation of these results is that the MLC stimulation (of the T cells) caused the reduction in IgE production, as was the case with the T cell mitogens.

Suppressor activity was also studied using another test. It has been shown that, on Con A stimulation of human lymphocytes in vitro, suppressor factors are produced which are soluble and can be demonstrated in the supernatants from the stimulated cells (1). The results of the suppressor tests with supernatants indicate that on Con A stimulation of lymphocytes factors are produced which are soluble and suppress IgE production in vitro. The suppression was weak. There was a tendency toward less inhibition from supernatants of suboptimally stimulated cells from AD patients compared with controls. This is in accordance with the hypothesis that dysfunction of the suppressor activity of the T cells may result in increased IgE production in atopic dermatitis. However, the number of tests, that could be performed, was not large

enough to permit any wide conclusions in this respect.

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