

Cellular immunity and IgE levels in atopic patients

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Twenty young adult atopic patients and their matched controls were studied. Spontaneously generated and Con-A-induced suppressor T cell functions as well as Natural Killer (NK) activity against K-562 target cells, measured in a short-time ³H-thymidine uptake, were evaluated. Suppressor T cell activity in the patients was more than 2 SD lower than that found in the controls and there was, contrary to expectation, a direct correlation between suppressor function and serum IgE levels. Atopic patients showed a statistically significant lower NK activity than normal controls when related to a low IL-2 production. Both facts inversely correlated with the concentration of IgE in serum. We concluded that atopic patients' vulnerability to viral infections may be due to defective NK activity. Suppressor T cell function is abnormal in these patients. Both defects could be due to a faulty immunoregulatory helper function.

Key words: atopy; IgE levels; NK activity; suppressor T cell function; interleukin-2.

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Several reports have suggested that atopic patients are more vulnerable to viral respiratory infections than normal individuals (14). The defense against viral infections is known to be mediated by the cell immune system. There has been great interest in studying the natural killer (NK) cell population because of their ability to kill target cells, especially tumor cells and virus infected cells (7). Moreover, the soluble factor interleukin-2 (IL-2) is known to be necessary for the activation of NK cells (9, 15).

Abnormal cellular suppressor function has been suggested to be the cause of the increased IgE levels in atopic patients (12); however, the results are controversial (14).

The present work examines aspects of the cellular immune system, which include suppressor cell functions, both spontaneously and Con-A-induced, total and active T rosettes with sheep red blood cells (SRBC), production of and response to IL-2, NK activity and mitogenic lymphocyte transformation. A group of 20 young adult atopic patients, their sex and age matched controls were studied and

the parameters were correlated with serum IgE levels.

MATERIAL AND METHODS

Twenty young atopic patients, 18 women and two men, mean age = 23 ± 5 years (range 18-30) and their healthy matched controls were studied. All subjects had positive immediate hypersensitivity reaction. No patients received steroid therapy, either during the study period or in the 3 months prior to the study. Salbutamol and theophylline were suspended at least 3 days before the study was performed. All patients had asthma, nine of them presented rhinitis, none had a recent history of atopic dermatitis, although two had had reactions to insect stings in their childhood.

IgE serum levels were measured by RIA, using the Phadebas IgE test kit.

For cellular immune studies 20 ml of peripheral blood was collected in heparinized syringes from each patient and control. Mononuclear cells (MNC) isolated by conventional Ficoll-

diatrizoate centrifugation were washed three times in RPMI-1640 supplemented with 5% immunoglobulin free Fetal Calf Serum (FCS), 1% L-glutamine, 100 UI/ml penicillin and 100 µg/ml streptomycin. Viable cells were counted using trypan blue dye exclusion, viability was always $\geq 97\%$. The preparation and counting of total and active T rosettes with SRBC were performed as described elsewhere (6). For mitogenic lymphocyte transformation MNC from each patient and control were stimulated in triplicate with optimal mitogenic concentration of Con-A, PHA and PWM in a 3-day culture, and 18 h before harvesting cells were pulsed with 1 µCi of methyl-3H-thymidine. Incorporation of the radiolabeled thymidine is reported in cpm and the stimulation index (SI) calculated as follows:

$$SI = \frac{\text{cpm of MNC with mitogen}}{\text{cpm of MNC without mitogen}}$$

The spontaneously generated and Con-A-induced suppressor functions were performed as described elsewhere (5). Briefly: 2×10^6 MNC were cultured either for 6 days without mitogens or 3 days with optimal mitogenic concentration of Con-A, at 37°C , 5% CO_2 in a humidified incubator. The cells were then washed with RPMI-1640. 1×10^5 cells in 100 µl after mitomycin-C treatment were co-cultured on microtiter plates with 2×10^5 fresh autologous MNC and 10 µl of optimal mitogenic concentration of PMW was added. Fresh MNC stimulated with PWM alone served as controls. Eighteen hours before harvesting, cells were pulsed with 1 µCi of 3H-thymidine. Percentage of suppression in both spontaneously generated and Con-A-induced was calculated as follows:

$$\% \text{ suppression} = 1 - \frac{\text{cpm of co-cultured cells}}{\text{cpm of fresh MNC alone}} \times 100$$

The IL-2 assay was performed as described elsewhere (2), i.e. in both patients and controls, MNC were incubated 24 h in RPMI with 5% FCS and 1% PHA in a 5% CO_2 , 100% humidity at 37°C ; cells were then washed three

times in RPMI, resuspended and incubated for another 24 h, and finally centrifuged at 2000 rpm for 20 min to collect the supernatants which were passed through 0.45 µm filters and stored at -20°C until used. IL-2 activity was measured in a human IL-2 addicted T cell line (LG-1). Four days after the last addition of lectin-free IL-2, 10^4 LG-1 cells were placed in 100 µl RPMI with 5% FCS in microcultured plates, the supernatants were added in three different dilutions, the plates were incubated at 37°C for 48 h. Twelve hours before harvesting 0.5 µCi of 3H-thymidine was added. Cellular proliferation induced by the IL-2-containing supernatants was expressed as the mean stimulation index (SI) calculated as follows:

$$SI = \frac{\text{mean cpm of cells cultured with supernatants}}{\text{mean cpm of cells cultured alone}}$$

NK activity against K-562 target cells was measured in a short-time 3H-thymidine uptake assay as described (8). 2.5×10^3 target cells (K-562) were co-cultured with different concentrations of the patients' and controls' MNC in 200 µl of RPMI with 5% FCS for 18 h with 1 µCi of 3H-thymidine. Killed cells were measured by the per cent of inhibition (PI) of 3H-thymidine incorporation calculated as follows:

$$PI = 1 - \frac{\text{cpm of co-cultured cells}}{\text{cpm of K-562 alone}} \times 100\%$$

Statistics analysis. The coefficient of correlation of Pearson (r) was used to calculate the correlation between IgE level and: suppressor T cell functions, NK activity, and IL-2 production.

RESULTS

There was no significant difference in the number of T cells measured in percentage of E rosettes between patient and control groups ($69 \pm 8\%$ vs $72 \pm 9\%$). The same relation was found in the active E rosette percentage.

The IgE levels in the control group were always below 80 KU/l and in the patient group

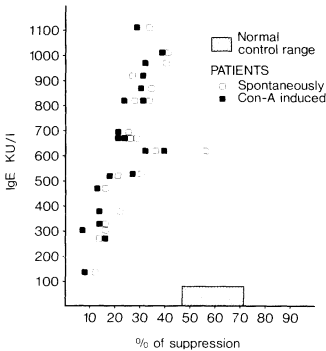


Fig. 1. Suppressor T cell function, both spontaneously generated and Con-A-induced, of atopic patients ($n = 20$) compared to normal controls.

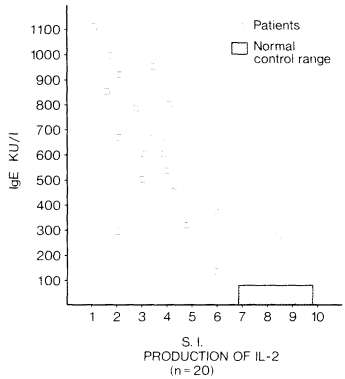


Fig. 2. Production of IL-2 by the MNC of peripheral blood of atopic patients ($n = 20$) compared to normal controls.

they ranged from 143 to 1100 KU/l with a mean value of 626 KU/l.

The mitogenic transformation assays showed no significant difference between the groups.

A comparison between patients and controls in the suppressor T cell functions, both spontaneously generated as well as Con-A induced, is presented in Fig. 1 and is related to the concentration of IgE in the sera. As can be observed all patients had a significantly lower suppressor function than the controls. Contrary to expectation, we found direct relation between suppressor function and the IgE levels ($r = 0.68$). The null hypothesis ($r = 0$) is rejected because the critical value at the 1% significance level was $= 0.561$. The difference is statistically significant at the 1% level. The confidence limit of the r value in the patient population was $0.192 \leq r \leq 0.897$ with a confidence of 99%.

NK activity was significantly lower in the atopic group compared to the controls ($P < 0.001$). The mean value in the relation 50 effector cells: 1 target cell was $55 \pm 7\%$ killed cells in the control group versus $14 \pm 3\%$ in the patient group.

As shown in Fig. 2 we found that a lower production of IL-2 in the atopic patients inversely correlated to the IgE levels ($r = -0.72$). The null hypothesis ($r = 0$) is rejected because the critical value at the 1% significance level was 0.561. The difference is statistically significant at the 1% level. The confidence limit of the r value in the atopic patients was $0.278 \leq r \leq 0.911$ with a confidence of 99%.

DISCUSSION

In the present study a significantly decreased suppressor T cell activity was found in the atopic patients compared with normal controls. This deficiency could explain the increased IgE levels in atopy. Although all atopic patients had a high IgE concentration in serum and low suppressor T cell function, the direct correlation between these two parameters reflects a more complicated defect in the immunoregulatory circuits in atopic patients.

On the other hand, atopic patients showed a statistically significant lower NK activity than normal controls, which was inversely correlated

to serum IgE level. This defect in NK activity may explain the vulnerability of atopic patients to viral infections (4, 11).

Our data are in agreement with others that have reported low suppressor cell activity (12) as well as normal active E-rosettes in asthmatic patients (10). However, the defects reported in this study could be explained by the lower production of IL-2 in atopic patients. IL-2 is synthesized by T lymphocytes that have helper and some immature surface markers (11). It has also been shown that IL-2 is an essential factor in the stimulation of NK activity (15). Moreover, it has recently been shown that helper T cells are needed for normal suppressor T cell function (5).

In our opinion the differences in the findings on NK activity between our work and that of others (13) could be due to two factors: 1) difference in the age of the patients, thus other factors could be involved, and 2) different methodology used in measuring the NK activity.

We conclude that suppressor T cell function and NK activity defects in asthmatic atopic patients may be due to a faulty immunoregulatory helper function.

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