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Quantitative and functional deficit of suppressor T cells in children with atopic eczema

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SUMMARY

Helper ($OKT4^+$) and suppressor ($OKT8^+$) T cells were enumerated in 16 children with severe atopic eczema. Compared to controls (median 1·8) the ratio of $OKT4^+/OKT8^+$ cells in the patients was significantly higher (median 2·65, $P < 0·002$). Functional suppressor activity in these patients was assessed by concanavalin A (Con A) activation and suppression of pokeweed mitogen (PWM) induced immunoglobulin production by plasma cells and Con A proliferation of T cells. In both assays a lack of suppression was shown (Con A/PWM, $P < 0·02$; Con A/Con A, $P < 0·05$). There was a significant inverse correlation between the helper/suppressor ratio and functional suppressor activity ($P < 0·01$). These results indicate that a defect of T cell regulation does exist in atopic eczema and if it is of primary pathogenic importance, immunotherapy to restore the balance may prove useful.

INTRODUCTION

It has been shown in rats that the production of IgE by plasma cells is under T cell contro (Okumura & Tada, 1971; Taniguchi & Tada, 1974). Inherently low IgE responder animals may b converted into high responders by treatment which preferentially affects suppressor T cells, i.e immunosuppressive drugs (Taniguchi & Tada, 1971), adult thymectomy (Okumura & Tada, 1971a and moderate doses of whole body irradiation (Tada, Taniguchi & Okumura, 1971). Th heightened response can be abrogated by transfer of syngeneic thymocytes (Okumura & Tad 1971b). The evidence therefore suggests that damping of the IgE response is by an antig non-specific T suppressor cell mechanism. Evidence for a similar mechanism in man circumstantial but allergy is significantly increased in immunodeficiency and very high levels of Ig may be associated with T cell deficiencies (Caplin *et al.*, 1977; Kikkawa *et al.*, 1973). It has bee suggested that defective T cell regulation may play some part in the development of allergy an indeed low numbers of T cells bearing Fc receptors for IgG (Tγ) (these include the suppress subpopulation) have been reported in atopic eczema (Canonico *et al.*, 1979; Jensen, Cramers Threstrup-Pederson, 1981). Recently monoclonal antisera which recognize the helper/inducer an suppressor/cytotoxic T cell subpopulations more accurately have been developed (Reinherz *et a* 1980). We have therefore studied children with atopic eczema to define their helper and suppress T cell ratios and correlated this with two functional assays measuring T suppressor cell activity

MATERIALS AND METHODS

Patients. Sixteen children, who were either inpatients at The Hospital for Sick Children

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attending the Dermatology outpatient clinic, were studied. All of the children suffered from severe atopic eczema and had elevated serum IgE levels (> three standard deviations for age). One patient had atopic asthma in addition. None of the patients had been treated with systemic steroids. The children were aged between 3 months and 13 years with a mean age of 5·5 years. Ethical approval had been obtained from the hospital and full informed consent was received from the parents. Blood was also obtained from 15 healthy non-atopic adults (mean age 32 years). Patients and control cells were always tested at the same time.

Lymphocyte separation. Ten millilitres of heparinized (preservative free) blood was diluted 1:2 with Hank's balanced salt solution (HBSS, Flow Laboratories), layered onto lymphocyte separation medium (Flow Laboratories) and centrifuged at 400 *g* for 20 min at 25°C. The layer of mononuclear cells at the interface were collected, washed twice with HBSS and resuspended at a concentration of 1×10^6 cells/ml in bicarbonate-buffered RPMI 1640 (Flow Laboratories) containing 2mM glutamine, 5 μg/ml gentamicin and 10% fetal calf serum (FCS, Flow Laboratories).

Measurement of T cell subsets. Subpopulations of T cells were estimated by indirect immunofluorescence. Monoclonal antisera recognizing all T cells (OKT3, Ortho diagnostics), helper/inducer (OKT4) and suppressor/cytotoxic (OKT8) subpopulations were used (Reinherz *et al.*, 1980). Five microlitres of the monoclonal reagents or HBSS was added to separate aliquots of 5×10^5 lymphocytes. The cells were incubated at 4°C for 30 min after which excess antibody was removed by washing three times with cold HBSS + azide (to prevent capping). Twenty microlitres of a second layer fluorescein labelled sheep anti-mouse Ig was then added to each aliquot and left to incubate for a further 30 min at 4°C. After three washes, wet slide preparations were made. Positively stained cells were counted using a Zeiss fluorescent microscope (150 cells were counted for each slide) and the results were expressed as a percentage after subtraction for non-specific staining.

Functional suppressor cell assays

Concanavalin A (Con A) suppression of pokeweed mitogen (PWM) stimulated immunoglobulin (Ig) synthesis (Layward, Levinsky & Butler, 1981).

(a) Cell culture. Cells were cultured in triplicate (200 μl volumes) in Linbro round bottomed microtitre plates at 37°C in 5% humidified CO_2 with or without PWM (Gibco Biocult, final dilution 1:200). Suppression of this PWM response was induced by co-culturing with 5 and 10 μg Con A (Sigma) and after 7 days the amount of Ig released into the supernatant was estimated.

(b) Competitive ELISA for total Ig production by cultures. An ELISA microtitre plate (Flow Laboratories) was coated overnight at 4°C with 5μg/ml of human Ig in 0·1 M carbonate/bicarbonate coating buffer, pH 9·5. After extensive washing with PBS, to remove unbound Ig, the remaining binding sites were blocked by incubating with 1% FCS in coating buffer for 1 hr at 37°C. The plate was then washed with PBS + 0·05% Tween 20 (Sigma) (PBS/Tween) and dried.

Meanwhile 50μl of culture supernatants were incubated with 50μl of peroxidase labelled anti-human Ig (DAKO, 1:400 final dilution) for 30 min at 37°C in plates which had been coated only with 1% FCS. Eighty microlitres of this mixture containing Ig/peroxidase labelled anti-Ig complexes were then transferred to the Ig coated ELISA plate and incubated for 1 hr at 37°C during which time any uncomplexed peroxidase labelled anti-Ig bound to the Ig coated plate. Unbound Ig was removed by extensive washing with PBS/Tween and bound peroxidase labelled anti-Ig was estimated by the addition of 100μl of substrate solution (40 mg *O*-phenylediamine in citrate/sodium dihydrogen phosphate buffer, pH 5). The enzymic reaction was carried out at 25°C in the dark and stopped after 30 min by the addition of 25μl of 4N sulphuric acid. The colour which developed during the reaction was read on a Titertek plate reader at the optimum wavelength of 492 nm. Sample concentrations were calculated by reference to a standard curve, range 1–50,000 ng Ig. Both samples and standards were set up in duplicate and mean values were calculated.

(c) Calculation of suppression.

$$\% \text{ suppression} = \frac{a - b}{a} \times 100$$

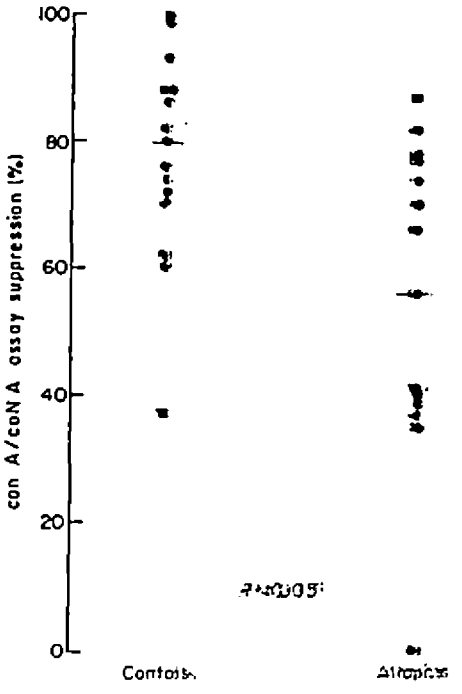


Fig. 3. Suppressor cell function measured by Con A suppression of Con A-induced T cell proliferation. Results expressed as percentages. Bars indicate median value.

$P < 0{\cdot}05$). These results indicate that the finding of reduced suppressor cells by monoclonal antisera (OKT8$^+$) in atopic eczema correlates with the functional abnormalities in suppressor activity measured by the two assays.

DISCUSSION

Our findings indicate that children with severe atopic eczema have a deficit in the number and function of suppressor T cells as compared to adult controls. For ethical reasons it was not possible to obtain blood from age matched children controls but data accumulated in our laboratory when testing children with suspected minor neutrophil immunodeficiencies has given equivalent results to those obtained in the adult controls. Furthermore, in studies where control data for children aged a few months to 17 years has been reported, the ratio of helper (OKT4$^+$) to suppressor (OKT8$^+$) was $1{\cdot}75 \pm 0{\cdot}3$ (2 s.d.) and this is very similar to that obtained in our adult controls (Siegel *et al.*, 1981; Leung, Rhodes & Geha, 1981).

There have been several studies of suppressor activity in atopy with reduced suppression reported by some (Strannegard, Strannegard & Dunér, 1978; Stingl, Gazze, Czarnecki & Wolff, 1981) but not by others (Martinez *et al.*, 1979). A reduction in T cells and return to normal numbers after successful desensitization has been shown in patients with asthma (Strannegard *et al.*, 1978). T cells include the suppressor subpopulation but not exclusively (Reinherz *et al.*, 1980) and furthermore the *in vitro* expression of T cells may be irreversibly blocked by IgG immune complexes (Pickering, Lum & Broder, 1978) so that the reduction observed may be a secondary effect of such complexes found in allergic individuals (Brostoff *et al.*, 1979). Our data using monoclonal antisera which accurately define the helper/inducer and suppressor/cytotoxic T cell subpopulations indicate that patients with severe atopic eczema do indeed have a deficit in the suppressor cells. These findings confirm those of Leung *et al.* (1978) but in addition our patients were shown to have functional suppressor cell deficiency. The significant inverse correlation of helper/suppressor T cell ratio with

Where

a = ng Ig produced by PWM stimulated cells

b = ng Ig produced by PWM stimulated cell + Con A (5 or 10 μg/ml).

Con A induced suppression of mitogen stimulation T cell proliferation (Con A/Con A assay) (Shou Schwartz & Good, 1976).

(a) Con A pre-treatment of lymphocytes. Suppressor T cells were generated by culturing lymphocytes for 24 hr at 37°C in 5% CO_2 in the presence of 10μg/ml Con A. At the end of the period the cells were washed twice in HBSS and resuspended in RPMI + 10% FCS. Control cells were treated in a similar manner but Con A was not present during the 24 hr incubation. The cells were given 2000 rad irradiation to prevent further cell division.

(b) Mitogen stimulation of responder cells. The suppressor and control cells were co-cultured with allogeneic responder cells from a healthy non-atopic donor. The ratio of suppressor/control cells to responder cells were kept at 1:2 (concentration of responder cells 1×10^6/ml). Triplicate cultures of cells with or without Con A (1μg/ml) were incubated in 5% CO_2 for 4 days at 37°C. Two microcuries of ^{3}H-thymidine (Radiochemical Centre, Amersham) were added for the final 24 hr of incubation. The cells were collected on the Titertek cell harvester and ^{3}H-thymidine incorporation was measured by liquid scintillation counting (LKB β counter). Counts were expressed as disintegrations per minute (d.p.m.) after correction for quenching and efficiency of the counter. The percentage of mitogen induced T cell transformation of responder cells which was inhibited by Con A induced suppressor cells, was calculated using the following formula:

$$\% \text{ suppression} = 1 - \frac{(Dmc - Dc)}{Dm - D} \times 100$$

Where:

Dmc = mean d.p.m. for suppressor cells and responder cells, mitogen added.

Dc = mean d.p.m. for suppressor cells and responder cells, no mitogen added.

Dm = mean d.p.m. for control cells and responder cells, mitogen added.

D = mean d.p.m. for control cells and responder cells, no mitogen added.

Statistics. Statistical analysis was by Mann–Whitney *U*-test and the Spearman Rank correlation test.

RESULTS

Both suppressor assays and T cell subset measurements were performed on 14 of the 16 patients. Insufficient blood was obtained from the other two patients, however, and T cell subsets only were measured in one, whilst T cell subset measurements and the Con A/Con A assay were performed on the other.

Measurement of T cell subsets

There were no significant differences in absolute lymphocyte numbers between the two groups nor did the eczema group differ from the control group in terms of total T cell (OKT3$^+$) percentages (control median 71%; eczema group median 65·6%). However, the eczema group had significantly lower percentages of suppressor/cytotoxic T cells (OKT8$^+$) (median 20·5%) than the control group (median 27·0%, $P < 0{\cdot}02$). There was no significant difference between the two groups for helper-inducer T cell (OKT4$^+$) subpopulation. When expressed as ratio of OKT4$^+$/OKT8$^+$ cells the eczema group had a significantly higher ratio (2·65:1·8, $P < 0{\cdot}002$) (Fig. 1).

Con A/PWM suppressor assay

In control subjects the addition of 10μg/ml Con A to PWM stimulated cultures greatly reduced Ig production with a median value of 88% suppression. In atopic patients this degree of suppression was lower giving a median value of 68%. The difference between these two groups was found to be highly significant ($P < 0{\cdot}02$) (Fig. 2). At 5 μg/ml Con A the degree of suppression was less in both groups and controls gave less consistent results with a median value of 80%. Nevertheless, the patients still had a significantly reduced degree of suppression and gave a median value of 52% ($P < 0{\cdot}02$).

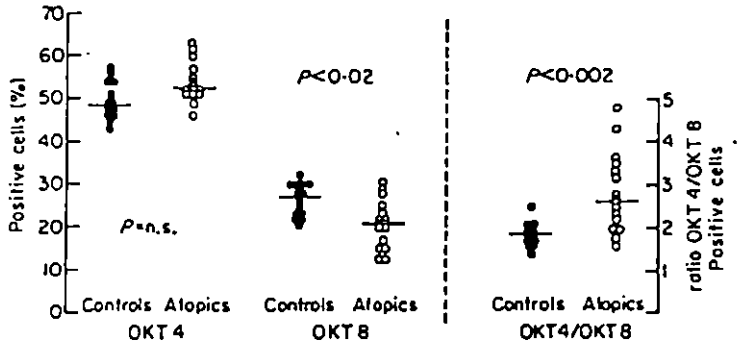


Fig. 1. Helper/inducer (OKT4$^+$) and suppressor/cytotoxic (OKT8$^+$) T cell subpopulations in controls and children with atopic eczema expressed as a percentage of mononuclear cells and as a ratio (OKT4$^+$/OKT8$^+$). Bars indicate median values.

Con A/Con A suppressor assay

We have previously established the optimal conditions for this assay and found that at a 1:2 suppressor/responder cell ratio consistent results are obtained. The control subjects had a median value of 80% suppression whereas the atopic eczema group had a significantly lower level of suppression with a median value of 50% ($P<0{\cdot}05$) (Fig. 3).

Correlation between the data

Results from the PWM assay (using 10 μg Con A) and the Con A/Con A assay were found to correlate significantly ($r_s=0{\cdot}5$, $P<0{\cdot}01$).

There was a significant inverse correlation between the ratio of OKT4 to OKT8 positive cells and the results from the PWM assay ($r_s=0{\cdot}49$, $P<0{\cdot}01$) and the Con A/Con A assay ($r_s=0{\cdot}3$,

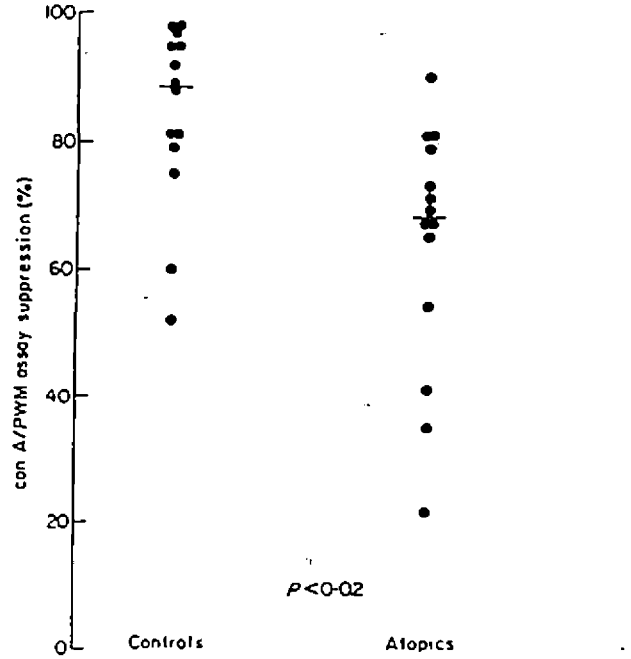
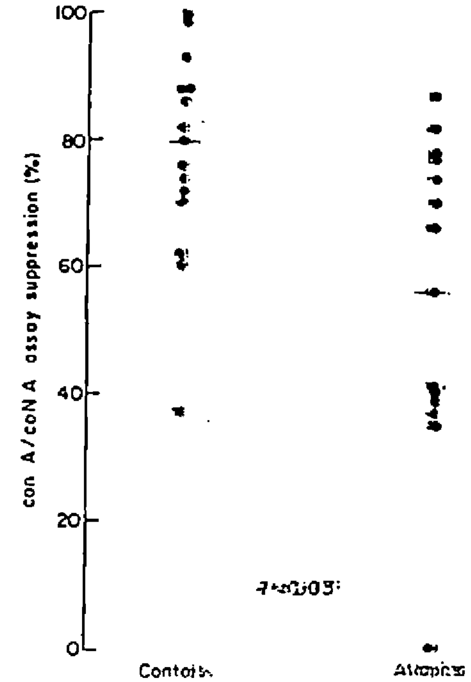


Fig. 2. Suppressor cell function measured by Con A suppression of PWM induced immunoglobulin production by plasma cells. Results expressed as percentages. Bars indicate median values.



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functional suppressor assays further emphasizes this point. The functional assays were antigen non-specific and suppressor cell activity was induced by activation with the mitogen Con A. In the mouse it has been shown that IgE helper T cells are more sensitive to non-specific suppressor T cell activity than IgG helper T cells (Karz *et al.*, 1974; Takatsu & Ishizaka, 1976). If a similar situation exists in man, the moderately depressed non-specific suppression seen in our study is likely to lead to preferential enhancement of IgE helper T cell activity and increased levels of IgE.

We do not know if our findings are of primary pathogenic importance in the development of atopic eczema or secondary to the disease. Prospective studies on 1 month old babies who later developed atopic disease, showed significantly lower total T cell numbers than control children, suggesting a primary defect in these children (Juto & Strannegard, 1979). Similarly, the improvement in antigen specific suppressor activity observed following successful desensitization for ragweed allergy suggests that this response can be modulated (Rocklin, 1981). Desensitization to the provoking antigens is not feasible in atopic eczema; improvement in clinical symptoms can be obtained by allergen avoidance especially offending foods, but in the majority of severe cases these are not known. We have recently shown that a synthetic pentapeptide thymic hormone (TP5, Ortho Pharmaceuticals) used for the treatment of immunodeficiency corrects imbalances of T cell subpopulations (Levinsky, Davies & Goldstein, unpublished observations). It seems reasonable to suggest that such immunotherapy may be of clinical benefit in patients with severe atopic eczema where trials of allergen avoidance have not shown improvement.

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