

Modulation of Fc receptors of thymus-derived lymphocytes by antigen E in ragweed-sensitive and ragweed non-sensitive subjects

K. S. ONG* and M. H. GRIECO

The R.A. Cooke Institute of Allergy, Medical Service, St. Luke's-Roosevelt Hospital Center and Department of Medicine, Columbia University, College of Physicians and Surgeons, New York, New York, U.S.A.

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Summary

In ragweed-sensitive and ragweed non-sensitive subjects the proportions of T lymphocytes bearing receptors for the Fc portion of IgG ($T\gamma$) and IgM ($T\mu$) were examined as obtained from the blood, and after treatment *in vitro* with ragweed antigen E or concanavalin-A.

The proportion of $T\gamma$ and $T\mu$ cells, from the peripheral blood of ragweed-sensitive and ragweed non-sensitive persons, untreated *in vitro*, were not statistically different. However when T cells from ragweed-sensitive subjects were exposed to ragweed antigen E, the $T\mu$ subpopulation was significantly increased ($P < 0.001$) without change in the $T\gamma$ population. The reverse change occurred when cells of ragweed non-sensitive subjects were treated with antigen E; there was an increase in the $T\gamma$ subpopulation ($P = 0.01$) but no change in number of the $T\mu$ cells. Cells from both the sensitive and non-sensitive groups showed increase in number of $T\gamma$ and reduced numbers of $T\mu$ cells when incubated with concanavalin A.

Since $T\gamma$ and $T\mu$ cells appear to have a regulatory function on B lymphocyte differentiation and antibody production, the pattern of responses of $T\gamma$ and $T\mu$ subpopulations *in vitro* to antigen E in ragweed-sensitive and ragweed non-sensitive subjects may reflect a difference in the cellular control of the immune response to ragweed antigen E.

Introduction

While it is well-established that the type I allergic reaction (Coombs & Gell, 1975) is elicited by mediators released as a result of the binding of allergens to mast cells or basophils sensitized with immunoglobulin E (IgE) (Orange & Austen, 1972; Kaliner & Austen, 1973) the cellular events leading to the increased formation of IgE have not been fully elucidated. However, it has been demonstrated from studies in animals

* Present address: Allergy-Immunology Division, Department of Pediatrics, University of California, College of Medicine, Irvine, California 92717, U.S.A.

Correspondence: Dr Michael H. Grieco, The R. A. Cooke Institute of Allergy, St. Luke's-Roosevelt Hospital Center, 428 West 59th Street, New York, New York 10019, U.S.A.

(Hamaoka, Katz & Benacerraf, 1973 and Okudaira & Ishizaka, 1973 and Tada & Okumura, 1971) that the synthesis of IgE antibodies is regulated by the interaction between thymus-derived (T) lymphocytes (functioning as helper and suppressor cells) and the IgE-forming non-thymus derived (B) lymphocytes. It seems possible that T cells in allergic subjects might be altered by having excessive helper function, diminished suppressor function or a combination of both. Recently, two subpopulations of human T cells have been identified (Moretta, Webb & Grossi *et al.*, 1977). T γ cells possess receptors for the Fc portion (FcR) of IgG and may function as suppressor cells, while T μ cells have receptors for the FcR of IgM and may help B cell differentiation and antibody production (Moretta *et al.*, 1977). Using these FcR as markers, it may be possible to detect differences in T cell subpopulations between allergic and non-allergic subjects, although recent studies suggest that Fc IgG and FcIgM do not appear to be markers for absolutely distinct T-cell subsets (Hayward, Layward & Lydyard, 1978; Pichler, Lum & Broder, 1978).

We sought to determine (1) whether there is a deficiency of the T γ subpopulation or an excess of the T μ subpopulation in the peripheral blood of allergic persons, and (2) whether these subpopulations would change in response to allergen challenge *in vitro*. The results presented here reveal no significant differences between allergic and non-allergic subjects in the respective percentages of T γ or T μ subpopulations in the peripheral blood. However, when T lymphocytes were exposed to antigen E (AgE) *in vitro*, ragweed-sensitive persons showed a great increase in T μ subpopulation, with no significant change in the T γ subpopulation; whereas in ragweed-nonsensitive persons AgE induced the reverse i.e., an increase in the T γ subpopulation and no change in the T μ subpopulations.

Patients and methods

Ten ragweed sensitive patients (ages 20–55) were selected on the basis of clinical symptoms of allergic rhinitis during the ragweed pollen season, positive reactions to intradermal tests with 100 protein nitrogen units (PNU)/ml of aqueous ragweed extract (Greer Laboratories, Lenoir, NC), and no previous immunotherapy. Ten subjects (ages 20–55) with no history of clinical symptoms during ragweed pollen season, negative reactions to intradermal tests of 100 PNE/ml of aqueous ragweed extract, and without any recent medical treatment were chosen as ragweed non-sensitive controls.

Thirty ml of peripheral venous blood was obtained from each study subject with a syringe containing 20 U/ml of preservative-free heparin (Sigma Chemical Co., St Louis, Mo.). Mononuclear cells were isolated on Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ, and Winthrop Laboratories, New York, NY) density gradient as described by Böyum (1968). Cells harvested from the interface were washed three times in calcium and magnesium-free Hank's balanced salt solution (Grand Island Biochemical Co., (GIBCO), Grand Island, NY). The remaining pellets were resuspended at a concentration of 4×10^6 /ml in RPMI-1640 medium (GIBCO) supplemented with 20% heat-inactivated foetal calf serum (GIBCO), 2.5 mmol HEPES buffer (GIBCO), 100 U/ml penicillin and 100 μ g/ml streptomycin (GIBCO). The resuspended mononuclear cells were depleted of phagocytes by incubating the cells with an equal volume of Lymphocyte Separator Reagent (Technicon, Tarrytown, NY), at 37°C for 1 hr. The iron-laden cells were then removed by centrifugation through Ficoll-Hypaque gradient. Purified T cell populations were obtained from

these macrophage-depleted lymphocytes by first rosetting with neuraminidase-treated sheep erythrocytes, and then separating the rosette-forming cells from the non-rosetting cells by centrifugation through Ficoll-Hypaque gradient (Parish *et al.*, 1974). The sheep erythrocytes were then lysed with 0.83% ammonium chloride (Siegal, Siegal & Good, 1976). The T cells were washed three times and resuspended in RPMI-1640 medium prepared as described above. The cell concentration was adjusted to $2 \times 10^6/\text{ml}$, and incubated overnight at 37°C in humidified air containing 5% CO_2 .

$\text{T}\gamma$ and $\text{T}\mu$ cells were identified by rosetting with ox red blood cells (ORBC) (Colorado Serum, Denver, Co). Anti-ORBC antibody was raised in New Zealand white rabbits by intraperitoneal immunization with ORBC according to the method of Harboe and Ingild. IgG and IgM fractions were prepared from pooled antisera by DEAE cellulose chromatography and Sephadex G-200 gel filtration, respectively. Both IgM and IgG fractions gave a single precipitating line on immunoelectrophoresis using monospecific goat anti-rabbit IgM or IgG. Fresh ox erythrocyte-antibody (EA) complex was prepared for each experiment by incubating 2% washed ORBC with an equal volume of optimally diluted IgM or IgG anti-ORBC antibody at room temperature for 90 min, then washing and resuspending at 1% concentration. $\text{T}\gamma$ and $\text{T}\mu$ rosettes were counted by mixing 100 μl of ox EA IgG or ox EA IgM with 100 μl of T cells incubated overnight, centrifuged at 200 g for 5 min and incubated at 4°C for 2 hr. Rosettes, defined as cells surrounded by three or more ORBC, were counted as the proportion of at least 200 lymphocytes in each tube without the examiner knowing the source of the cells (blindfold fashion).

Prior to incubation of the isolated T cell population, various concentrations of ragweed AgE (Research Resources Branch, NIAID, Bethesda, Md) were added to different tubes to achieve final concentrations of 0.01, 0.1, 1, 10, 50, 100 μg per/ml of RPMI-1640 medium. In an initial study, these concentrations produced no apparent dose-response difference in the T cell subpopulations. In subsequent studies, therefore, only concentrations of 0.1 $\mu\text{g}/\text{ml}$ and 50 $\mu\text{g}/\text{ml}$ were used as the respective low and high doses of allergens. From each study subject, one tube of T cells was incubated with concanavalin A (con-A) at a final concentration of 60 $\mu\text{g}/\text{ml}$. This concentration has been shown to induce suppressor T cells. (Shou, Schwartz & Good, 1976). Another tube of T cells was incubated with purified protein derivatives of *Mycobacterium tuberculosis* (PPD) (Connaught Laboratories, Willowdale, Ontario, Canada) at a final concentration of 1 $\mu\text{g}/\text{ml}$. Statistical analysis was performed using the Student's *t* test.

Results

There was no significant difference in the proportion of $\text{T}\gamma$ and $\text{T}\mu$ cells in untreated preparations from ragweed-sensitive and ragweed non-sensitive subjects (Tables 1 and 2).

Table 1 summarizes the mean percentage $\pm$ standard error of the mean (s.e. mean) for $\text{T}\gamma$ cells in ragweed-sensitive and ragweed non-sensitive subjects before and after incubation with antigen E and con A. None of the blood samples from 10 ragweed-sensitive individuals showed an increase in $\text{T}\gamma$ cells of more than 1% after overnight AgE incubation. The $\text{T}\gamma$ subpopulation of the ten ragweed non-sensitive subjects was significantly higher in tubes incubated with AgE ($P < 0.01$). In this group only one of the ten blood samples did not demonstrate an increase in $\text{T}\gamma$ cell proportion

Table 1. Percentage T cell counts in ragweed-sensitive and ragweed non-sensitive subjects before and after incubation with AgE and con A (mean $\pm$ s.e. mean)

Antigen E	0 μ g/ml (n = 10)	50 μ g/ml (n = 10)	P Value
Ragweed-sensitive	5.40 $\pm$ 0.54	5.20 $\pm$ 0.66	Not significant
Ragweed non-sensitive	5.00 $\pm$ 0.67	9.40 $\pm$ 1.75	< 0.01

Antigen E	0 μ g/ml (n = 7)	0.1 μ g/ml (n = 7)	P Value
Ragweed-sensitive	5.00 $\pm$ 0.72	5.14 $\pm$ 0.86	Not significant
Ragweed non-sensitive	4.14 $\pm$ 0.46	8.29 $\pm$ 0.78	< 0.001

Concanavalin A	0 μ g/ml (n = 8)	60 μ g/ml (n = 8)	P Value
Ragweed-sensitive	5.13 $\pm$ 0.64	11.38 $\pm$ 1.49	< 0.001
Ragweed non-sensitive	4.25 $\pm$ 0.41	8.38 $\pm$ 1.48	< 0.01

n = number of subjects.

after *in vitro* incubation with 50 μ g/ml AgE. When incubated with 0.1 μ g/ml of AgE, this blood sample however had a 100% increase of T γ cells.

Table 2 summarizes the T μ proportions and indicates that incubation of T cells with AgE in ragweed-sensitive subjects significantly increased the number of T μ cells at concentrations of either 50 μ g/ml or 0.1 μ g/ml AgE ($P < 0.001$). The T μ cell proportion did not increase after incubation with 50 μ g/ml in only one patient; however, when T cells from this blood sample were incubated with 0.1 μ g/ml of AgE, there was an increase of 83%. No significant changes in T μ subpopulations were observed in ragweed non-sensitive subjects after *in vitro* exposure to AgE. Cell viability as determined by trypan blue exclusion test was more than 92% with both T cell subpopulations incubated with 0.1 μ g/ml or 50 μ g/ml AgE.

A significant enhancement of the T γ subpopulation and decrease of the T μ subpopulations was detected in both ragweed-sensitive and ragweed non-sensitive subjects after incubation of T cells with 60 μ g/ml con-A and indicated the responsiveness of T γ cells in allergic subjects to a mitogen.

Incubation with PPD was performed using T cells from nine subjects with negative reactions (five were ragweed-sensitive and two were ragweed non-sensitive) to intradermal PPD 5-TU. No changes were produced in T γ or T μ subpopulations in the PPD-negative group. Mean $\pm$ s.e. mean of T γ cell count was 4.67 $\pm$ 0.50 when incubated without PPD, and 4.33 $\pm$ 0.55 when incubated with PPD; the T μ cell count was 27.33 $\pm$ 2.52 without and 25.22 $\pm$ 2.75 with PPD (P not significant). In the PPD-positive group, the T γ cell proportions were not significantly changed after incubation with PPD (mean $\pm$ s.e. mean were 4.0 $\pm$ 1.0 and 4.5 $\pm$ 0.5 when incubated with and without PPD respectively). However, the T μ cell proportions increased significantly after incubation with PPD (mean $\pm$ s.e. mean were 36.3 $\pm$ 2.1 without and 46.8 $\pm$ 2.2 with PPD, $P < 0.01$).

Table 2. Percentage T μ cell counts in ragweed-sensitive and ragweed non-sensitive subjects before and after incubation with AgE and con A (mean $\pm$ s.e. mean)

Antigen E	0 μ g/ml (n = 10)	50 μ g/ml (n = 10)	P Value
Ragweed-sensitive	31.90 $\pm$ 3.51	47.00 $\pm$ 5.70	< 0.001
Ragweed non-sensitive	30.40 $\pm$ 2.14	28.30 $\pm$ 3.21	Not significant
Antigen E	0 μ g/ml (n = 7)	0.1 μ g/ml (n = 7)	P Value
Ragweed-sensitive	28.57 $\pm$ 3.40	44.00 $\pm$ 5.25	< 0.001
Ragweed non-sensitive	28.43 $\pm$ 2.64	28.00 $\pm$ 3.06	Not significant
Concanavalin A	0 μ g/ml	60 μ g/ml	P Value
Ragweed-sensitive	(n = 7) 28.57 $\pm$ 3.40	(n = 7) 13.14 $\pm$ 1.47	< 0.01
Ragweed non-sensitive	(n = 8) 29.75 $\pm$ 2.64	(n = 8) 10.88 $\pm$ 1.47	< 0.01

n = number of subjects.

Discussion

The present study demonstrated that the baseline proportion of T γ and T μ cells in the peripheral blood of ragweed-sensitive patients was not significantly different from that of ragweed non-sensitive subjects. This finding contrasts with a recent report (Canonica, Mingari & Melioli, 1979) which showed a significantly reduced T γ subpopulation in atopic patients with asthma or severe rhinitis. Our studies, however, were performed during mid-winter and spring, when ragweed-sensitive subjects were asymptomatic with regard to ragweed exposure. We have found in a group of patients with atopic dermatitis that the proportion of T γ subpopulation was significantly reduced only in those with acute exacerbation of the disease. Therefore, the alteration of T cell subpopulations may correlate with the severity of disease rather than atopy *per se*.

The increased proportion of T μ cells in the ragweed-sensitive group after incubation with AgE may reflect 'helper' cells generated in the secondary response to antigen, since T μ cells were also increased with the T cells of PPD-positive subjects incubated with PPD regardless of whether they were ragweed-sensitive or non-sensitive. T μ cells were unchanged with cells from PPD-negative subjects after antigen incubation as were T μ cells of ragweed non-sensitive subjects. The enhancement of T γ cells in the ragweed-nonsensitive group after AgE challenge may reflect 'suppressor' cell activity but the same was not observed with cells of PPD-negative subjects when their T cells were incubated with PPD *in vitro* regardless whether they were ragweed-sensitive or nonsensitive.

Since ragweed AgE has been reported to behave as a mitogen for lymphocytes (Buckley *et al.*, 1977) one may question whether the changes in T γ cells in

ragweed-nonsensitive subjects are the same as those induced by con-A (Gupta & Good, 1978). It could be considered that ragweed-sensitive subjects have generally defective T γ responses to mitogen. That this is not the case is suggested by the demonstration that incubation with con-A induced increased T γ subpopulations in both ragweed-sensitive and non-sensitive groups. The lack of increase in T γ cells in the ragweed-sensitive subjects seems to be specific for ragweed AgE. Moreover, two of the ten ragweed non-sensitive subjects had symptoms of allergic rhinitis and positive reactions to skin tests for grass and trees but not for ragweed. When their T cells were incubated with AgE, they showed changes similar to those of other non-allergic subjects with enhancement of the T γ proportion. This suggests that the increase in the T γ subpopulation is not determined by the overall allergic status but rather by the sensitivity of the individual to the particular allergen used for *in vitro* challenge.

In view of the fact that purified preparations of AgE may be contaminated with endotoxin lipopolysaccharide (LPS), T cells from four ragweed-sensitive and four ragweed-nonsensitive subjects were studied similarly, using *Escherichia coli* LPS (kindly provided by Dr James Watson of the Microbiology Department, College of Medicine, University of California at Irvine) at a concentration of 0.1 and 1 μ g/ml. In both ragweed-sensitive and nonsensitive subjects, there was no significant differences in the T γ or T μ cell proportion following exposure to LPS at either the 0.1 or 1 μ g/ml concentrations.

The mean proportion of T μ cells in this study is low when compared to others in the literature. We believed that this resulted from aggregation of IgM in the batch of rabbit anti-ORBC serum that was used. However, it is unlikely that the increased T μ cells observed in ragweed-sensitive subjects is due to improvement of rosette formation in the presence of AgE since, under identical conditions, no increment of T μ cells was found in ragweed non-sensitive subjects.

However, the enhanced response of T γ cells in the ragweed non-sensitive subjects after *in vitro* AgE challenge is extremely interesting. It is possible that the enhanced T γ cells function that inhibits IgE production and therefore protects the subject from clinical illness when exposed to ragweed allergens, cannot be determined without further functional analysis of the T γ subpopulation. This finding seems very comparable to the response pattern of responder and non-responder mice and guinea pigs to synthetic copolymers, where the non-responders react to a particular antigen challenge by enhanced suppressor T cells which hinder the immune response against that antigen (Kapp *et al.*, 1974). It is well established that this immunological responsiveness is determined by an immune-response regulating gene or genes (Ir genes) linked to the major histocompatibility complex (McDevitt & Chinitz, 1969). The possible existence of an Ir-antigen E gene or genes in ragweed allergy patients has been suggested by Levine *et al.* (1972) on the basis of family studies in human ragweed allergy. If this is the case, then a lack of a suppressor T γ cell response in ragweed-sensitive subjects could be due either to an Ir-antigen E gene or genes depressing the T γ response or, alternatively, depression of another gene or genes normally eliciting the suppressor response.

The functional analysis of these changes of T cell subpopulations in ragweed-sensitive and non-sensitive subjects is currently under study. Changes in the proportion of the T γ and T μ subpopulations in response to ragweed AgE exposure may reflect a difference in the cellular control of the immune response to ragweed antigen E in allergic subjects.

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