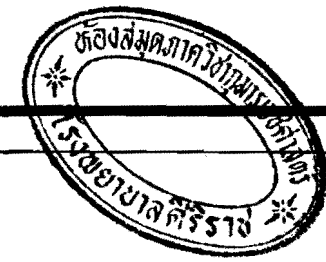
Asian Pacific Journal of Allergy and Immunology (1994) 12 : 111-115



Immunological and Clinical Evaluation during a 12 Month Period of Immunotherapy

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SUMMARY **Serum IgE and IgG4 were evaluated in twenty adult allergic rhinitis subjects during a 12-month immunotherapy (IT) course against common inhalant allergens. The selection criteria for IT were the result of a prolonged history of allergic diseases and a positive skin test for common inhalant allergens. Twenty non-atopic adults served as the control group. By using enzyme-linked immunoassay a normal range of IgE and IgG4 were 78±40 IU/ml and 180±54 μg/ml, respectively. The changes in IgE and IgG4 levels were compared with significant improvements of symptoms and drug consumption. The clinical and laboratory responses to IT were considered good in 8 cases (40%), moderate in 7 cases (35%), and poor in the remaining 5 cases (25%). A regression analysis revealed a negative simple linear correlation between elevated level of IgG4 and diminished level of clinical symptom scores during 12 months of IT (Pearson's r=-0.7548). The serum IgG4 level after one year IT predicted change in clinical symptom scores.**

Immunotherapy has been used in an attempt to block immune response to inhalant allergens since 1911 when Noon and Freeman[1] attempted to modify the symptom of their patients with allergic rhinitis and asthma. Immunotherapy or hyposensitization is a form of treatment that attempts to increase the threshold level for symptom appearance following exposure to the aeroallergen. This altered degree of sensitivity results either in induction of a new antibody, the so-called "blocking antibody" or in a decrease in reaginic antibody.[2] A variety of immunologic changes have been demonstrated that may in part be responsible for the modulation of allergic reactions by immunotherapy.The efficacy of antigen-specific parenteral IT in amelioration of the symptoms of hay fever during the pollen season has been well established in multiple controlled clinical trials.[3-5] In the study by Lichtenstein *et al*[6] of ragweed allergic patients, those individuals with high levels of IgG blocking antibodies more often demonstrated low symptom scores on symptom-score diaries kept during the ragweed season. The effectiveness appears to depend on the potency of the allergenic material used in the treatment: the dose schedule and the treatment regimen.[7] It is likely that the selection of patients is also important.

Immunologically, a variety of changes have been demonstrated to be responsible for the relief of allergic symptoms: (1) rise in serum IgG "blocking antibodies" (2) suppression of the usual seasonal rise in serum IgE antibodies that follows environmental exposure, and a slow decline during several years in the level of specific IgE antibodies.[8] There is still much speculation on the poorly understood mechanism of effective IT. Considerable attention has been devoted to the role of IgG subclasses, both in the pathogenesis of anaphylaxis (IgG1 and IgG4) and in relation to the clinical outcome.

In this study, changes in the levels of serum IgE and IgG4 during a 12-month IT course were examined. The problems of reliable clinical

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assessment, the use of sensitive assays for the measurement of antibody levels, and the possible role of IgG4 as a blocking antibody were addressed.

MATERIALS AND METHOD

Patient selection

Twenty non-atopic adults served as the control group for normal ranges of serum IgE and IgG4 levels (mean ± SD) 78 ± 40 IU/ml, and (mean ± SD) 180 ± 54 μg/ml, respectively. Twenty adult patients comprising 13 females and 7 males, aged 17 to 47 years (mean 34.5 years) were the experimental group. The criteria for patient selection were a result of prolonged history of allergic rhinitis with failure of medical treatment, selection a positive skin test for common inhalant allergens, a lack of previous IT, a serum IgE level above 158 IU/ml, (mean + 2 SD) and a serum IgG4 level below 72 μg/ml, (mean-2 SD).

Reagents

Allergenic extract standardized mite *Dermatophagoides farinae* was provided by Greer Laboratories. Ten-fold dilution series were performed in normal saline containing 0.4% phenol.

Treatment schedules[9]

The selected patients were received allergenic extract, started with a dose of 1:100,000 dilution weight by volume (w/v). The dosage was increased every 7 days until the maintenance dose was achieved. Maintenance doses of 0.50 ml of a 1:100 (w/v) allergenic extract were given at weekly intervals (Table 1).

Table 1. Schedule of dosage increases for immunotherapy.[9]

Extract concentration (w/v)	Dosage (ml)[a]
1:100,000	0.05, 0.1, 0.2, 0.3, 0.4, 0.5
1:10,000	0.05, 0.1, 0.2, 0.3, 0.4, 0.5
1:1,000	0.05, 0.1, 0.2, 0.3, 0.4, 0.5
1:100[b]	0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5 (maintenance dose)

a = Doses were given every 7 days.
b = Maintenance dose of 0.5 ml of a 1:100 (w/v) allergenic extract.

Serologic measurements

Immunoglobulin E and subclass IgG4 were assayed by "sandwich" enzyme immunoassay (EIA). Microtitre wells were coated with goat anti-human IgE (Sera-Lab) or anti-sheep subclass IgG4 (ICN-Immu-Mark) depend on their specification. For IgE assay, standard IgE (Behring) equivalent to 7,140 IU/ml (calibrated against WHO standard 68/341) was diluted with PBS (phosphate buffered saline) pH 7.2 (10.0; 25.0; 50.0; and 200,0 IU/ml). The British standards for human immunoglobulin E (IgE Code 75/502) were absorbed and included in the IgE assay as control sera. Dilutions were made with PBS buffer to 10.0 and 100.0 IU/ml. Sera from normal (n = 20) and atopic patients (n = 20) were diluted 1:10 or 1:20 and dispensed 100 μl each in duplicate into microtitre wells previously coated with IgE antibodies, blocked with 1% BSA (bovine serum albumin) and incubated 37°C for 2 hours. After thorough washing of the wells to remove unbound proteins, anti-human IgE peroxidase conjugate was added to each well and incubated for 1 hour. Excess (unbound) conjugated was removed by further washing of the wells. The bound conjugate was then visualized using o-phenylenediamine (OPD) and hydrogen peroxide which gives a yellow product within 30 minutes. The amount of conjugate bound and color produced was proportional to the concentration of IgE in the specimens, according to the reading at 450 nm within 2 hours.

Table 2. Clinical symptoms and drug scores.

No symptom	0
Episodes of sneezing	1
Nasal blockage	1
Rhinorrhea	1
Pruritus of the nose	1
No medication	0
Nasal spray with beclomethasone	3
Oral antihistamine	3
Oral prednisolone	4

For subclass IgG4 assay, standard IgG4 (ICN) calibrated against WHO/IUIS SPS 01 was diluted with PBS at 1.4, 2.8, 5.6, 11.0, 23.0, 45 and 90.0 μg/l. Human control sera calibrated against WHO/IUIS SPS 01 were also diluted 1:300 (96 μg/l) and 1:30,000 with PBS and 100 μl pipetted in duplicate together with standard into the previously coated microtitre well and incubated at 37°C for 2 hours. The washing procedures were as for the IgE assay. After washing the antihuman IgG4 peroxidase conjugate were added and incubated 37°C for another 2 hours. Washing procedures were repeated and the wells dried before adding the substrate (OPD) and hydrogen peroxide. The plates were left in the dark at room temperature for 30 minutes before dispensing 50 μl of 4N sulfuric acid into each well

for reaction termination. The optical density (OD) of each well was read at 450 nm within 2 hours.

Clinical parameters

Clinical symptoms and drug scores of the patients had been recorded one month before the treatment was started, and at the end of 3, 6 and 12 month periods of IT, as shown in Table 2.

Serum samples were collected from all patients before and after the 3, 6 and 12 months of IT, patients' responses were classified as good, moderate and poor according to the serum IgG4 level. In the good response group, patients had serum IgG4 above 234 μg/ml, (>mean + SD) whereas patients in the moderate-response group had serum IgG4 levels ranging from 126 to 234 μg/ml, (mean ± SD) and patients in the poor-response group had serum IgG4 below 126 ug/ml, (< mean-SD).

Statistical methods

Comparison of mean values was achieved by ANOVA to avoid the use of multiple *t*-tests. Two-parameter comparisons were carried out with a combination of scatter diagrams, simple linear regression, and correlation analysis, linearizing data where it was appropriate by using logarithmic transformation.

RESULTS

The overall levels serum antibodies of the patients (n = 20) before IT were: IgE = 516 IU/ml and IgG4 = 49 μg/ml.

Serum IgE

Table 3 shows in the good response group, statistically significant ($p. < 0.05$) progressive decreases of serum IgE during the first 3 and 6 months. However, after 6 months, there was slight improvement. In the moderate-response group, changes in serum IgE level became statistically significant ($p < .0.05$)

Table 3. Serum IgE level during 0, 3, 6, and 12 months of immunotherapy.

Time (months)	Serum IgE* (IU/ml)		
	Good	Moderate	Poor
0	383.63[a]	571.86[a]	652.00[a]
3	294.38[b]	471.86[b]	443.00[b]
6	169.38[c]	311.14[b]	371.60[b]
12	105.13[c]	193.29[c]	267.50[b]

*Different superscripts in the same column indicate statistically significant differences.

Table 4. Serum IgG4 level during 0, 3, 6, and 12 months of immunotherapy.

Time (months)	Serum IgG4* (μg/ml)		
	Good	Moderate	Poor
0	56.50[a]	40.71[a]	48.76[a]
3	67.75[a]	52.14[a]	57.66[a]
6	129.75[b]	83.61[b]	72.13[b]
12	249.75[c]	163.33[c]	94.45[c]

*Different superscripts in the same column indicate statistically significant differences.

Table 5. Clinical symptom scores during 0, 3, 6, and 12 months of immunotherapy.

Time (months)	Clinical symptom scores*		
	Good	Moderate	Poor
0	7.75[a]	7.43[a]	6.80[a]
3	5.75[b]	7.75[b]	5.60[a]
6	2.75[c]	2.29[c]	2.60[b]
12	0.38[d]	1.29[c]	1.00[b]

*Different superscripts in the same column indicate statistically significant differences.

during the first 6 months of IT. In the poor-response group, after 3 months of IT, statistically significant decreases ($p < 0.05$) in serum IgE level occurred. After 6 and 12 months of IT, the IgE level decreased but this was not statistically significant.

Serum IgG4

Table 4 shows changes in serum IgG4 level that followed a similar pattern in all three groups; these were not statistically significant during the first 3 months of IT. Progressive changes took place after 3 months of treatment: in terms of quantity, the amount of serum IgG4 was statistically significantly increased ($p < 0.05$) in all three groups.

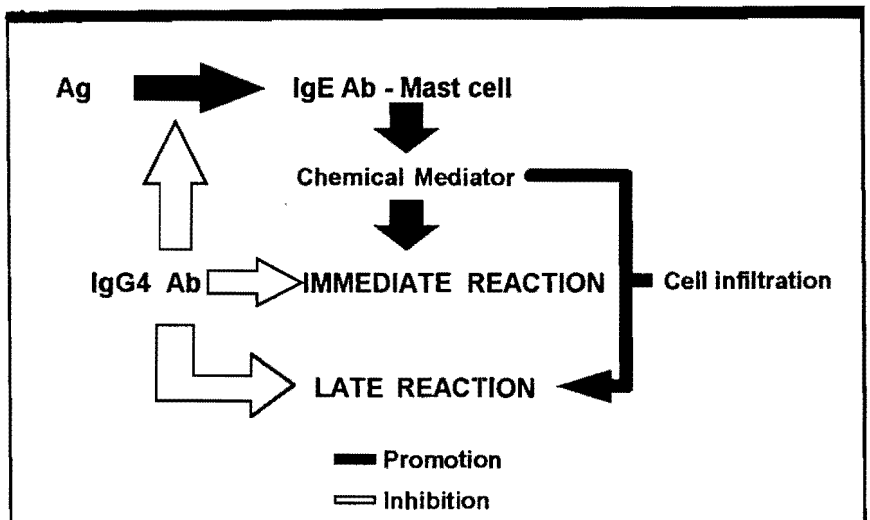

Fig. 1. Machanism of action of blocking antibody IgG4.

Clinical evaluation

Based on the clinical symptoms and drug scores, changes in scores in the good-response group during the first 3 months are shown in Table 5. These progressive changes persisted for the next 6 and 12 months. In the moderate-response group, there were changes in scores during the first 3 and 6 months. In the poor-response group, improvement was noted after 6 months and persisted until the end of 12 months. In all three groups, there was statistically significant improvement ($p < 0.05$) in clinical symptom scores after 6 months of IT.

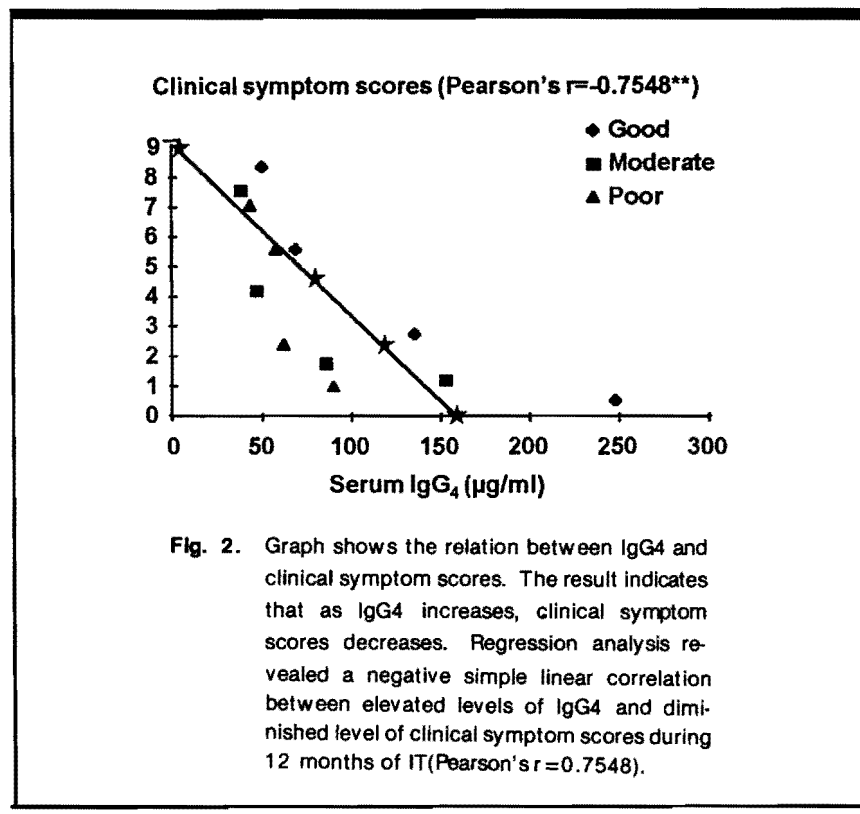

Fig. 2. Graph shows the relation between IgG4 and clinical symptom scores. The result indicates that as IgG4 increases, clinical symptom scores decreases. Regression analysis revealed a negative simple linear correlation between elevated levels of IgG4 and diminished level of clinical symptom scores during 12 months of IT(Pearson's r=0.7548).

DISCUSSION

Controversy exists about the role of IgG and its subclasses in the response to IT. The role of IgG4 in the pathogenesis of allergic diseases is unclear, but it possesses functions that might either mediate short-term anaphylaxis, or protect against allergic reactions. It appears unlikely that IgG4 is important in inducing prolonged release of mediators,[8] since we have demonstrated that patients who produced IgG4 showed significant clinical improvement. Indeed, it has recently been demonstrated that serum with a high level of IgG4 could block IgE-mediated diseases.[8,10] As a result, the mechanism of IgG4 blocking antibody can be deduced as shown in Fig. 1.

Our results indicate that as IgG4 increases, clinical symptom scores decrease. Regression analysis revealed a negative, simple linear correlation between elevated levels of IgG4 and diminished level of

clinical symptom scores during 12 months of IT (Pearson's r = −0.7548) (Fig. 2). Our findings are also compatible with the concept of IgG4 acting as a blocking antibody. Before starting IT, the mean of IgE levels and IgG4 levels were 516 IU/ml and 49.0 μg/ml, respectively, which proved to be helpful in the selection of patients, since a recent study concluded that immunological criteria for allergic diseases were IgE >300 IU/ml and IgG4 <60 μg/ml.[11] Our study supports these criteria. Responses to IT were different in each patient and also depended upon the individual environment, so we could not predict individual clinical improvement. Therefore, by using clinical symptom scores, serum IgE and serum IgG4, patients' responses can be helpful in guiding the treatment of incoming patients, since knowledge of his or her response patterns will indicate the appropriate treatment that should be administered.

ACKNOWLEDGEMENTS

This work received financial support from the Pramongkutklao Hospital Fund.

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