

IgG Subclass Antibody Response in Grass Pollen-Allergic Patients Undergoing Specific Immunotherapy

Prognostic Value of Serum IgG Subclass Antibody Levels Early in Immunotherapy

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All four subclasses of IgG antibodies to timothy grass pollen extract were measured by a three-layer immunoradiometric assay in sera from 20 grass pollen-allergic patients who underwent specific immunotherapy in a 3-year prospective study. Both IgG1 and IgG4 antibody levels rose significantly during the first 8 weeks of immunotherapy. IgG1 antibody level passed its peak (median 5.4 U/ml) after 12 weeks. At this time, the ratio between the medians of IgG1 and IgG4 antibodies was 2.25. IgG4 antibody level reached its peak (median 11.6 U/ml) just before termination of immunotherapy. At this time IgG1/IgG4 ratio was 0.43. Two years after the end of immunotherapy, IgG1 and IgG4 antibody levels were 0.0 and 1.8 U/ml in median, respectively. The amounts of IgG2 and IgG3 antibodies detected in the sera were less than 1.6 U/ml and were considered insignificant. Preseasonal serum IgG1 and IgG4 antibody levels did not correlate significantly with symptom scores in the subsequent season. Serum IgG4 level obtained after 12 weeks of immunotherapy was significantly correlated to symptom score in the third season, i.e. the season just after termination of therapy ($r_s = 0.529$, $t = 2.567$, $P = 0.02$). In this work, a serum IgG4 antibody level higher than 8.0 U/ml after 12 weeks of therapy predicted poor clinical result at the end of immunotherapy with 100% sensitivity and 87% specificity. An IgG4/IgG1 ratio greater than 1.0 after 12 weeks' therapy had the same predictive value.

Key words: allergic asthma; allergic rhinitis; hay fever; IgG subclass antibodies; immunoradiometric assay; immunotherapy.

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CLINICAL ASPECTS

The results presented in this paper indicate that a high IgG4 antibody level early in the course of immunotherapy with timothy grass pollen extract may predict a poor clinical response to the treatment.

During immunotherapy of patients with allergic rhinitis and asthma a rise in specific serum IgG is seen in most patients (11, 13, 22). Traditionally, it has been assumed that these IgG antibodies are protective (2, 4, 11), and they have been named "blocking antibodies". Although a correlation between specific serum IgG level and clinical protection against symp-

toms on allergen exposure has been demonstrated in patients immunized with insect venoms (9, 10), we (5, 21) and others (11, 13) have been unable to demonstrate such a relation in patients immunized with inhalant allergens. As IgG antibodies of different subclasses may play different roles in the response to immunotherapy, measurement of the IgG sub-

class antibody response during such therapy may give more valuable information than determination of the total IgG antibody production (2, 4). However, due to lack of suitable reagents and methods (2), only a few attempts to study the nature and role of the IgG subclass antibody response during immunotherapy have been performed (4, 8, 18), and most recent studies have focused solely on the IgG4 response.

We have recently developed a solid-phase immunoradiometric assay, named TIRA (6), which permits reliable intra-individual and inter-individual comparison of the magnitude of the IgG subclass antibody response to grass pollen allergens. In the present work we have applied this method to study the IgG subclass antibody response in sera from grass pollen allergic patients who underwent specific immunotherapy (hyposensitization) for 3 years in a prospective study (19, 20, 21, 22). We describe the changes in serum IgG subclass antibody levels, their mutual relations, and the relation between IgG subclass and IgE antibody levels during and up to 2 years after the end of immunotherapy. The relations between the preseasonal serum IgG subclass antibody levels and the seasonal symptom score, and between the initial rise in serum IgG subclass antibody concentrations and symptom score in the first season after termination of therapy, were also assessed.

MATERIAL AND METHODS

Patients, immunotherapy procedure, and symptom score registration

The study design, patients, immunotherapy procedure, and method of symptom score registration have been described in detail elsewhere (19). Only the 20 patients immunized with a partially purified timothy grass pollen extract (Alutard® SQ 225, ALK, Copenhagen, Denmark) were studied in the present work. Alutard SQ 225 consists of 20 out of the approximately 30 timothy (*Phleum pratense*) antigens, adsorbed to Al(OH)₃, and is a biologically stan-

dardized extract. Immunotherapy was carried out weekly from January 1978 until the season, June 1978, beginning with 2 biological units (BU). The maintenance dose, reached in week 12, was 10,000 BU, or else the highest tolerated. From grass pollen season 1978 the interval between injections was increased to 4 weeks. In the season the dose was reduced to 1/3–1/4. Serum samples were collected at week 0 (before treatment, January 1978), and after weeks 8, 12 (preseason 1, 1978), 30 (postseason 1), 64 (preseason 2, 1979), 82 (postseason 2), 116 (preseason 3, 1980), 134 (postseason 3), 168 (1 year after end of treatment), and 220 (2 years after end of treatment).

Symptoms from eyes, nose and lung were recorded daily during the season according to the score system described in (19). Symptom scores used in the present work also include the medicine used by the patients (19).

Quantitation of serum IgG subclass antibodies to timothy pollen extract

Serum IgG subclass antibodies to timothy pollen extract were measured by a three-layer immunoradiometric assay (TIRA) as described elsewhere (6). In brief, 0.2 ml serum diluted 1:40, or more, were incubated for 2 h at 37°C with a nylon ball covalently coated with timothy pollen extract (Aquagen® SQ 225, ALK). The balls were then incubated with 0.2 ml mouse monoclonal antibodies to human IgG subclasses (Seward Laboratory, London, England) for 2 h at 37°C. The monoclonal antibodies were used in the dilutions 1:1,000, 1:500, 1:24,000, and 1:24,000 of anti-IgG1, anti-IgG2, anti-IgG3, and anti-IgG4, respectively. (At these dilutions, the signal (cpm bound) is the same for equivalent amounts of antibodies in the 4 subclasses (6)). Finally, the balls were incubated overnight at room temperature with about 40 ng (specific activity 4 mCi/mg) of ¹²⁵I-labelled affinity-purified rabbit anti-mouse IgG, and counted for 1 min in a gamma-counter. Specific binding was calculated by subtraction of non-specific binding, obtained with pooled normal human serum, from total binding. The assay was calibrated by use of dilutions of a human reference

serum (J.N. (6)), arbitrarily ascribed 100 U IgG4 antibody to grass pollen/ml undiluted serum. The antibody concentrations were expressed in units/ml undiluted serum (U/ml). Values below 1.6 U/ml (corresponding to about twice the total binding observed with pooled normal human serum) were considered insignificant. The assay was found to be specific for antibodies to grass pollen (6). The monoclonal antibodies reacted specifically with their complementary IgG subclass proteins, but neither with IgG proteins of other subclasses, nor with IgA, IgE or IgM proteins (6). All determinations were done in duplicate. The coefficient of variation of counts for duplicates ranged from 0 to about 25%, omitting obvious outliers. For most samples it was in the range 5–10%. The mean of the counts (corrected for non-specific binding) was used for interpolation on the standard curve.

The inter-assay coefficient of variation of serum IgG subclass antibody levels (in U/ml) was from 18 to 27% in the present study ($n = 7$ (6)).

Measurement of serum IgE antibodies to timothy pollen extract

Serum IgE antibodies to timothy pollen extract were measured by Al-RAST (17, 20). As allergosorbent was used timothy pollen extract (Aquagen SQ 225) coupled to $\text{Al}(\text{OH})_3$ (Alhydrogel®, Superfos, Vedbæk, Denmark). Bound IgE was detected by use of ^{125}I -labelled rabbit anti-human IgE (RAST Isotop®, Pharmacia, Uppsala, Sweden). The results were expressed as sorbent units/ml undiluted serum (SU/ml) (20). Most of these results have previously been presented (21), but are included in the present paper for comparison with the results of the IgG subclass antibody determinations.

Statistics

The significance of the observed changes in antibody concentrations in sera obtained at two consecutive samplings was assessed by the Wilcoxon matched-pairs signed-rank test (15). The

same test was applied for comparison of the magnitude of IgG1 and IgG4 antibody concentrations in sera obtained at the same sampling. The mutual relation between IgG1 and IgG4 antibody concentrations, between IgE antibody and IgG subclass antibody concentrations, and between preseasonal serum antibody concentrations and seasonal symptom score was tested by calculation of Spearman's rank correlation coefficient, r_s (15). The relation between serum antibody concentrations obtained after 3 months of immunotherapy and the symptom score in the third grass pollen season was assessed in the same way. Significance of the rank correlation coefficients was tested by calculation of a t -value as described in (15). Changes or correlations with a two-sided stochastic probability equal to or less than 0.05 were considered statistically significant.

RESULTS

Three patients had significant concentrations of serum IgG1 to timothy pollen before start of immunotherapy. The changes in serum IgG1 antibody level in the individual patient during and after end of immunotherapy are shown in Fig. 1. Significant amounts of IgG2 or IgG3 antibodies to timothy were not found in any of the serum samples. One patient had significant concentration of serum IgG4 to timothy pollen before start of therapy. The changes in serum IgG4 antibody concentration in the individual patient during and after end of therapy are shown in Fig. 2.

Serum IgE antibody rose significantly during the first 8 weeks of immunotherapy (Table 1), reached a maximum before the first grass pollen season, and decreased significantly from postseason 1 to preseason 2. During season 2 a small, but significant increase was seen. After end of immunotherapy, during season 3, serum IgE antibody fell significantly, but rose again before season 4, whereupon the serum IgE antibody level remained unchanged.

Serum IgG1 antibody rose significantly during the first 8 weeks of immunotherapy (Table 1), passed a peak after 12 weeks, and declined significantly during the first pollen season. A

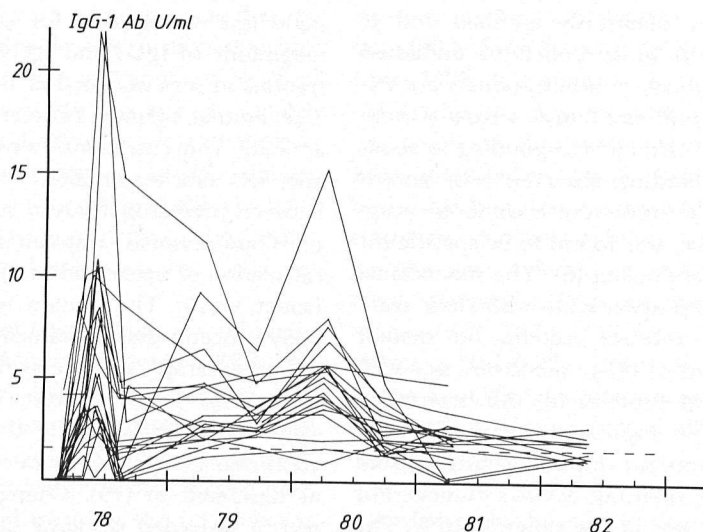


Fig. 1. IgG1 antibody concentration in sera from 20 grass pollen-allergic patients undergoing specific immunotherapy from January 1978 to beginning of the grass pollen season 1980. Values below 1.6 U/ml (dotted line) were considered insignificant. *Abscissa*: Time (year). *Ordinate*: Antibody concentration in U/ml undiluted serum.

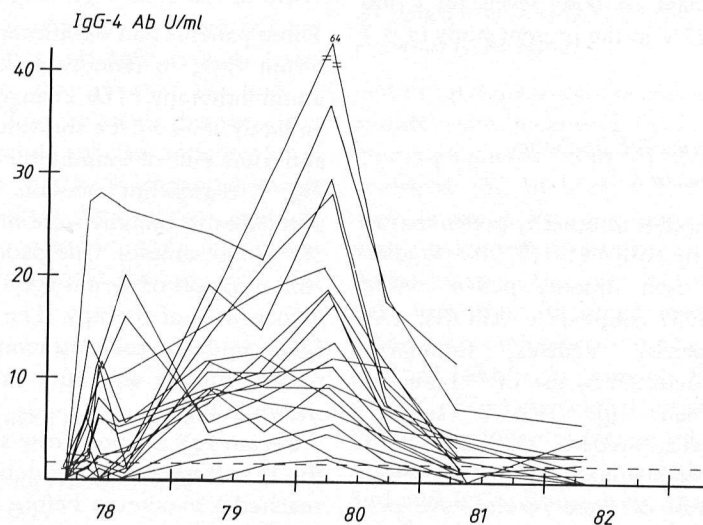


Fig. 2. IgG4 antibody concentration in sera from 20 grass pollen-allergic patients undergoing specific immunotherapy from January 1978 to beginning of the grass pollen season 1980. Values below 1.6 U/ml (dotted line) were considered insignificant. One patient did not have significant amounts of IgG4 antibody at any time. *Abscissa*: Time (year). *Ordinate*: Antibody concentration in U/ml undiluted serum.

non-significant increase was seen from post-season 1 to pre-season 2. IgG1 antibody decreased significantly during season 2, but increased again up to season 3. After end of

immunotherapy IgG1 antibody level decreased during season 3, after which no further significant changes were seen.

Serum IgG4 antibody increased significantly

during the first 8 weeks of immunotherapy (Table 1). No significant change was observed during the first pollen season. IgG4 antibody level rose significantly from postseason 1 to pre-season 2, and from postseason 2 to pre-season 3, where it reached its peak. When immunotherapy was discontinued, serum IgG4 antibody level fell significantly during season 3, and from postseason 3 to pre-season 4.

After 64 weeks of immunotherapy serum IgG4 antibody level became significantly higher than serum IgG1 antibody level. This significant difference between the levels of the two antibody classes was seen up to postseason 3 (Table 1).

During the first 30 weeks of immunotherapy serum IgE and IgG1 antibody concentrations were significantly correlated (Table 2). From week 64 up to and including week 116 serum IgG1 and IgG4 antibody concentrations were significantly correlated. At the end of the immunotherapy (week 116), serum IgE, IgG1 and IgG4 antibodies were all mutually correlated.

Correlations of serum IgE and serum IgG subclass antibody levels with clinical effect

Medians (and 0.25–0.75 quantiles) of symptom scores in the grass pollen seasons 1978, 1979, and 1980 were 113 (28–197), 129 (0–315), and 1 (0–76), respectively.

Seasonal symptom scores were not correlated to pre-seasonal levels of serum IgE, IgG1, or IgG4 antibodies during the immunotherapy (Table 3). Symptom scores in the third season (i.e. the season just after the end of immunotherapy) were significantly correlated to serum IgG4 antibody concentrations obtained after 12 weeks of immunotherapy (Table 3). Pretreatment levels of serum IgE antibodies were not correlated to symptom scores in the third season (results not shown).

DISCUSSION

By use of an assay which demonstrates absolute subclass specificity and measures IgG antibodies

Table 1

Medians (and 0.25–0.75 quantiles) of serum IgE antibody (SU/ml) and serum IgG subclass antibody (U/ml) concentrations before (week 0), during (weeks 8–116) and up to 2 years after end of immunotherapy (weeks 134–220)

	Week No.	Serum IgE Ab	Serum IgG1 Ab	Serum IgG4 Ab
Season 78	0 ^a	21 (5–46)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
	8 ^a	147* (65–314)	3.1* (2.3–5.7)	2.1* (0.0–5.8)
	12 ^a	280* (116–515)	5.4* (3.2–10.2)	2.4 (0.0–10.2)
Season 79	30 ^a	227 (54–363)	0.8* (0.0–4.1)	2.6 (0.0–5.6)
	64 ^b	8* (4–30)	3.2 (2.4–4.8)	8.8* (4.4–11.6) [§]
	82 ^b	31* (8–46)	2.9* (2.4–3.8)	9.4 (5.7–12.4) [§]
Season 80	116 ^a	15 (3–49)	5.0* (3.7–5.6)	11.6* (6.0–19.8) [§]
	134 ^a	5* (2–12)	2.4* (1.0–3.2)	4.9* (2.6–9.6) [§]
	168 ^a	14* (5–39)	2.0 (0.0–2.6)	0.7* (0.0–3.0)
Season 81	220 ^c	12 (6–56)	0.0 (0.0–2.0)	1.8 (0.0–2.6)

^a Sample size $n = 20$.

^b Sample size $n = 19$.

^c Sample size $n = 11$.

* The antibody levels in the samples concerned are significantly different from those in the preceding samples in the same antibody class.

[§] IgG1 antibody concentrations are significantly different from IgG4 antibody concentrations in the samples obtained at the time indicated.

Table 2
Correlations between serum IgE, IgG1 and IgG4 antibody concentrations during (weeks 8–116) and up to 2 years after end of immunotherapy (weeks 134–220)

	Week No.	x	y	n	r _s	t
Season 78	8	IgE	IgG1	20	0.466	2.235*
		IgE	IgG4		0.047	0.200
		IgG1	IgG4		−0.128	−0.548
	12	IgE	IgG1	20	0.467	2.241*
		IgE	IgG4		0.232	1.012
		IgG1	IgG4		0.197	0.853
	30	IgE	IgG1	20	0.457	2.180*
		IgE	IgG4		−0.067	−0.285
		IgG1	IgG4		−0.232	−1.012
Season 79	64	IgE	IgG1	19	−0.217	−0.917
		IgE	IgG4		−0.151	−0.630
		IgG1	IgG4		0.516	2.484*
Season 80	82	IgE	IgG1	20	0.084	0.358
		IgE	IgG4		0.146	0.626
		IgG1	IgG4		0.498	2.436*
	116	IgE	IgG1	20	0.559	2.860*
		IgE	IgG4		0.517	2.562*
		IgG1	IgG4		0.597	3.157*
	134	IgE	IgG1	20	0.091	0.388
		IgE	IgG4		0.308	1.374
		IgG1	IgG4		0.127	0.543
Season 81	168	IgE	IgG1	20	0.171	0.736
		IgE	IgG4		0.625	3.397*
		IgG1	IgG4		0.195	0.844
	220	IgE	IgG1	11	0.282	0.882
		IgE	IgG4		0.375	1.214
		IgG1	IgG4		−0.114	−0.344

n: Sample size.
r_s: Spearman's rank correlation coefficient.
t: t-value, calculated as described in (15), for the null hypothesis that r_s is zero.
* Two-sided probability for the observed t-value is ≤ 0.05.

of all subclasses with almost equal sensitivity (6), we have shown that parenteral immunization of allergic patients with a partially purified timothy grass pollen extract results in production of IgG1 and IgG4 antibodies only. Previously, Devey et al. (4) have found antibodies to grass pollen in all four subclasses during and after preseasonal immunotherapy with a crude mixture of 12 grasses. However, the mean titres of IgG1 and IgG4 antibodies were significantly

greater than those of IgG2 and IgG3 antibodies. Their assay showed both subclass specificity and equipotency, but was only semi-quantitative and of low sensitivity. In fact, the mean log₂ titers for IgG2 and IgG3 antibodies did not exceed 1, and we consider the observations of these authors in agreement with our results. Using an assay which showed subclass specificity, but slightly different sensitivity for the 4 subclasses, van der Giessen et al. (8) found a predominant IgG4

Table 3

Correlation of preseasonal serum IgE and IgG subclass antibody concentration with seasonal symptom score

X		Y	n	r_s	t
IgE	78	SS 78	20	-0.117	-0.498
IgG1	78	SS 78	20	0.038	0.161
IgG4	78	SS 78	20	0.218	0.948
IgE	79	SS 79	19	0.081	0.334
IgG1	79	SS 79	19	0.124	0.516
IgG4	79	SS 79	19	0.417	1.892
IgE	80	SS 80	19	0.451	2.085
IgG1	80	SS 80	19	0.054	0.223
IgG4	80	SS 80	19	0.409	1.847
IgE	78	SS 80	19	0.197	0.828
IgG1	78	SS 80	19	0.132	0.547
IgG4	78	SS 80	19	0.529	2.567*

X: Preseasonal serum antibody level in the class and year indicated.

Y: Symptom score (SS) in the grass pollen season in the year indicated.

n: Number of patients.

 r_s : Spearman's rank correlation coefficient.t: t-value, calculated as described in (15), for the null hypothesis that r_s is zero.* Two-sided probability for the observed t-value is ≤ 0.05 .

Table 4

Association of high level of IgG4 antibody (> 8.0 U/ml) after 12 weeks of immunotherapy with poor clinical result (symptom scores > 76) in season 3

	SS ≤ 76	SS > 76	
IgG4 ≤ 8.0	13	0	13
IgG4 > 8.0	2	4	6
	15	4	19

Serum IgG4 antibody > 8.0 U/ml after 12 weeks of immunotherapy has (4/4) 100% sensitivity and (13/15) 87% specificity as predictor of a poor clinical result, defined as symptom score > 76 in the first grass pollen season following the end of the therapy.

antibody response in patients undergoing immunotherapy for 2 years with an aqueous grass pollen extract of undescribed composition. Low levels of IgG1 and IgG2 antibodies, which only increased slightly during treatment, were also observed in some sera. In contrast, Zanussi &

Pastorello (18) found significant increases in IgG antibodies of all four subclasses in patients treated with either an aqueous 3-grass mixture or an alginate-conjugated 2-grass mixture. The data presented in this preliminary report did not permit evaluation of the specificity or relative sensitivity of the assay for the four IgG subclasses.

Our results confirm the observation made by Aalberse et al. (1), that prolonged immunization with at least some allergens may result in an IgG4 dominated antibody response. We have further shown that persisting antigen stimulation is necessary for maintenance of a high IgG4 antibody level. In this context it is of interest that autoantibodies to thyroglobulin in patients with Hashimoto thyroiditis (16), to factor VIII in hemophilic patients (3), and to *Schistosoma* antigens in chronically infected patients (12), also predominantly are of the IgG4 subclass. Thus, it is tempting to speculate that one biological function of the IgG4 antibody subclass, which neither activates complement via the classical pathway nor binds to cells (14)

(with the exception of mast cells and basophils), may be to protect the organism against inappropriate immunological reactions during persisting antigen exposure.

Apparently, IgG4 antibodies may function as sensitizing antibodies in some patients (2, 7). However, the lack of correlation between pre-seasonal serum IgG4 antibody level and seasonal symptom score (Table 2) indicates that IgG4 antibodies did not contribute substantially to symptoms in the present study.

The statistically significant correlation between the IgG4 antibody level after 12 weeks of immunotherapy and the symptom score in the season following termination of therapy, indicates that the magnitude of the early rise in the IgG4 antibody concentration may be a valuable indicator for the final outcome of immunotherapy with timothy grass pollen. Thus, if treatment failure is defined as symptom score greater than 76 (the 0.75 quantile), IgG4 antibody level higher than 8.0 U/ml after 12 weeks of therapy predicted treatment failure with 100% sensitivity and 87% specificity (Table 4). An IgG4/IgG1 ratio greater than 1.0 had the same prognostic significance (results not shown). The fact that high IgE response to one of the major allergens (Ag 19) of timothy grass pollen extract, and increase in skin threshold during the first 12 weeks of immunotherapy, also were predictive of good clinical response in the subsequent pollen seasons (22) makes it unlikely that the observed statistically significant correlation between the early rise in IgG4 antibody level and clinical response is a coincidence without immunological foundation. To elucidate whether this correlation is a more general phenomenon during immunotherapy with grass pollen and other allergens, we recommend frequent measurements of the IgG subclass antibody levels during the early course of immunotherapy studies.

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