

Quantitative IgE- and IgG-subclass responses during and after long-term ragweed immunotherapy

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We studied the quantitative responses of short ragweed (RW)-pollen-specific serum antibodies in 22 patients with RW immunotherapy (IT) and in a different set of 31 patients, 16 of whom stopped RW IT after more than 5 years of treatment. Serum was assayed before and after season, 1 year before and 1 and 2 years after starting IT, and 1 year and 2 years after stopping IT. RW pan-IgG, RW IgG1, and RW IgG4 were measured by ELISA, and RW IgE by RAST. Absolute quantities of RW IgG1 and RW IgG4 in reference sera were estimated by least-squares multiple regression analysis of 223 sera with the equation $RW \text{ pan-IgG} = RW \text{ IgG1} + RW \text{ IgG4}$. IgG1 is dominant in the early immune response of IT and disappears relatively slowly when IT is stopped. In contrast, IgG4 appears in significant quantities only after prolonged IT and disappears rapidly when IT is stopped. The apparent average half-life of RW IgG4 (9 months) was significantly shorter than that of RW IgG1 (29 months) ($p < 0.001$). Before IT, mean RW IgE rose 180% ($p < 0.01$) during the RW pollination season (August to November). This seasonal rise in RW IgE was ablated after IT from 1 year up to 8 years, but returned the year after IT was stopped. After 2 years of IT, the RW IgG1 and IgG4 levels were significantly correlated with RW IgE ($r = 0.94$ and 0.81 ; $p = 0.0001$ and 0.005). (J ALLERGY CLIN IMMUNOL 1992;89:519-29.)

Key words: Ragweed, IgE, IgG subclasses, immunotherapy, allergy, human

Previous studies in IT have demonstrated that a variety of immunologic changes may, in part, be responsible for relief of allergic symptoms. These include (1) a marked rise in serum IgG "blocking antibodies" that are mainly restricted to subclasses IgG1 and IgG4,¹⁻⁹ (2) a rise initially in serum IgE antibodies, followed by a failure of the customary rise after natural seasonal exposure, and ultimately by a slow decline to pretreatment levels or even lower levels after several years,¹⁰⁻¹⁷ (3) other immunologic changes, including a reduction of in vitro lymphocyte responsiveness to

Abbreviations used

RW:	Short ragweed
IT:	Immunotherapy
RW pan-IgG:	RW-specific serum IgG antibody
RW IgG1:	RW-specific serum IgG1 antibody
RW IgG4:	RW-specific serum IgG4 antibody
RW IgE:	RW-specific serum IgE antibody
1-yr M-IT:	One year of immunotherapy with moderate dose of RW
2-yr H-IT:	One year of immunotherapy with moderate dose of RW followed by another year IT with high dose of RW
BSA:	Bovine serum albumin

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allergen,¹⁸ an inhibition of the seasonal increase of eosinophil cationic protein,¹⁹ a decrease of histamine-releasing factor production,²⁰ and a fall in sensitivities of target cells (basophils and/or mast cells) or end-organ responsiveness to the offending allergen.^{12, 13, 17, 21-24}

In our continuing studies of the mechanism of protection provided by IT, we wanted quantitative data on antigen-specific IgG-subclass responses as well as

responses of IgE antibodies during and after a long course of IT. We describe our method of quantifying the absolute amounts (micrograms per milliliter) of RW IgG1 and RW IgG4 and the quantitative levels of RW IgG1, RW IgG4, and RW IgE before IT, during 2 years of active IT, and for 2 years after long-term IT was stopped in patients allergic to RW-pollen extract.

MATERIAL AND METHODS

Subjects and treatment

Twenty-two patients with RW hay fever were recruited in Baltimore before the 1985 RW season, based on medical history and a positive intradermal skin test (>10 mm wheal to a concentration of ≤ 10 PNU/ml of RW extract). Beginning in February 1986, patients received RW (Greer Laboratories, Lenoir, N.C.) IT up to a maintenance dose of 2 μ g of *Amb a* 1 (formerly antigen E) during 3 months (1-yr M-IT). In February 1987, 12 patients withdrew; the remaining 10 patients had the strength of their maintenance level injections increased gradually to 20 μ g *Amb a* 1 per injection (2-yr H-IT). Serum was obtained before and after season, 1 year before, and at 1 and 2 years after receiving IT.

Thirty-one patients with hay fever participated in a randomized study of stopping IT after the 1985 season. Each patient had been receiving maintenance IT for more than 5 years with RW plus one or more other allergoids, including grass pollen, plantain, or oak. The subjects were skin tested with RW and randomized into two groups of similar sensitivity. One group ($N = 16$) continued treatment with booster injections every 6 weeks of 100 allergoid units with all relevant allergoids except RW. Another group ($N = 15$) continued unchanged allergoid treatments that included RW. Serum samples were obtained before and after season in 1985 and at 1 and 2 years after IT was stopped.

ELISA for RW pan-IgG, RW IgG1, and RW IgG4

Reagents. Lyophilized short RW extract (lot No. L56-85, 217 μ g of *Amb a* 1 per milliliter) was supplied by Research Resources Branch of National Institute of Allergy and Infectious Diseases (National Institutes of Health, Bethesda, Md.). Affinity-purified alkaline phosphatase-conjugated goat antimouse IgG (code No. 650) was purchased from Tago, Inc. (Burlingame, Calif.). Mouse monoclonal antihuman pan-IgG (clone HP 6064, lot No. 3598R) was purchased from Unipath, Inc. (Bedford, U.K.). Mouse monoclonal antihuman IgG1 (ascites fluid, clone No. HP 6069, lot No. 102688) and antihuman IgG4 (ascites fluid, clone HP 6025, lot No. 111085) were kindly provided by Dr. Robert G. Hamilton. The monoclonal antihuman IgG4 was later purified from ascites by a diethylaminoethyl cellulose in our laboratory. The specificity and immunoreactivity of these mouse monoclonal antihuman IgG1 and IgG4 antibodies were confirmed in an International Union of Immunological Societies/World Health Organization collaborative study. No cross-reactivity with either immunoglob-

ulins of other animals or other human subclasses was observed.^{25, 26}

The absolute quantity of RW pan-IgG in reference serum No. 6630 (15.4 μ g/ml) was estimated by a *Staphylococcus* A solid-phase RIA elution technique, as previously described.²⁷ The absolute quantity of RW IgG1 in reference serum No. 7278 and quantity of RW IgG4 in reference serum No. 6630 were estimated by least-squares multiple-regression analysis of 223 sera, as described below.

Assay procedure. An indirect ELISA, described previously,^{28, 29} with some modification was used to measure RW pan-IgG, RW IgG1, and RW IgG4. The precision and reproducibility of these assays have been described previously.²⁹ The interassay mean coefficients of variations of interpolated doses were 0.18 ± 0.01 (mean $\pm$ SEM) for RW IgG1, 0.17 ± 0.01 for RW IgG4, and 0.11 ± 0.01 for RW pan-IgG. Briefly, polystyrene microtiter plates (PGC Scientific Laboratories, Gaithersburg, Md.) were coated with RW allergen, 0.02 μ g per well, in 100 μ l of 0.1 mol/L of acetate buffer (containing 1.0 mol/L of NaCl, pH 6.5) overnight at 4° C and then washed three times with ELISA buffer (0.01 mol/L of NaPO₄, 0.15 mol/L of NaCl, and 0.05% Tween 20, pH 7.4). One hundred microliters of a 1:100 or greater (for the RW pan-IgG assay) and 1:25 or greater (for the RW IgG1 and RW IgG4 assay) dilutions of either reference serum or unknown sera was added, respectively, to antigen-coated wells in duplicate. All dilutions of serum beyond the initial dilution were made in normal serum (1:100 diluted in 0.2% BSA ELISA buffer) for the RW pan-IgG assay or in 0.2% BSA ELISA buffer for the RW IgG-subclass assays. After an overnight incubation at 4° C, the plates were washed three times with ELISA buffer, and 100 μ l of mouse monoclonal antihuman pan-IgG diluted 1:10,000, or antihuman IgG1 diluted 1:1000, or antihuman IgG4 diluted 1:3000 in 0.2% BSA ELISA buffer was added to each well. After a 2-hour incubation at 37° C, the plates were washed three times with ELISA buffer, and 100 μ l of affinity-purified alkaline phosphatase-conjugated goat antimouse IgG, diluted 1:4000 for RW pan-IgG, 1:1000 for RW IgG1, or 1:3000 for RW IgG4 in 0.2% BSA ELISA buffer, was added to each well. After plates were washed three times with ELISA buffer, 100 μ l of *p*-nitrophenylphosphate substrate was added and incubated overnight at 4° C. The reaction was stopped by the addition of 100 μ l of 1 N NaOH, and the optical density was read at 410 nm on an automated MR700 microplate reader (Dynatech Laboratories, Alexandria, Va.). The levels of RW antibody in unknown sera were calculated by interpolation from a reference dilution curve. The sensitivities for the present assays are 1 ng/ml for RW pan-IgG and RW IgG1, and 0.1 ng/ml for RW IgG4.

RAST for RW IgE

RW IgE was measured with a standard agarose-based RAST.³⁰ Briefly, RW (lot No. 156-44, Greer Laboratories) was insolubilized to CNBr-activated Sepharose CL-4B beads (Pharmacia, Piscataway, N.J.) and then sequentially incubated with either reference serum No. 5089 or unknown sera and ¹²⁵I-labeled rabbit antihuman IgE (made and purified

in our laboratory). The absolute value of RW IgE in reference serum No. 5089 was estimated by a RAST elution technique.³¹ Bound radioactivity was quantitated in a gamma counter. The RW IgE values of unknown sera were interpolated from the reference serum dilution curve.

Statistical analysis

For ELISA and RAST immunoassays, the dilution curve of reference serum was mathematically fit by a three-parameter spline function, as previously described.³² The level of RW antibody in unknown serum was calculated from the curve function. For performance assessment, the ELISA precision profiles were calculated.³³

Analysis of data was performed with CLINFO (Scripps clinic) software provided by The Johns Hopkins Clinical Research Center. For group comparison, Student's paired or unpaired *t* tests were used. Pearson's correlation coefficients were used throughout.

RESULTS

Quantitation of RW IgG1 and RW IgG4

RW pan-IgG in micrograms per milliliter and RW IgG1 and RW IgG4 in units per milliliter were measured from 223 serum samples. Previous studies have demonstrated that during IT, antigen-specific pan-IgG response is restricted to IgG1 and IgG4.^{3, 4, 7} For estimation purposes, it was assumed that RW pan-IgG content of each serum was equal to the sum of RW IgG1 and RW IgG4. Because the absolute quantity of RW pan-IgG was independently determined by elution technique,²⁷ the values for antibody units of RW IgG1 and RW IgG4 were estimated by least-squares multiple-regression analysis with the equation $\text{RW pan-IgG} = \text{RW IgG1} + \text{RW IgG4}$. The best-fit solution with 223 sera estimated the RW IgG1 content in reference No. 7278 to be 18 $\mu\text{g/ml}$ and 7 $\mu\text{g/ml}$ for RW IgG4 in reference No. 6630. As observed in Fig. 1, there was a strong correlation between RW pan-IgG and the sum of RW IgG1 plus RW IgG4 in 223 sera ($r = 0.91$; $p < 0.0001$).

RW IgG1 and IgG4 responses

The RW IgG1 and RW IgG4 responses to IT are presented in Tables I and II and Figs. 2 and 3. Geometric mean RW IgG1 levels increased from 1.13 $\mu\text{g/ml}$ before IT to 3.14 $\mu\text{g/ml}$ at 1 year of IT and then to 4.77 $\mu\text{g/ml}$ at 2 years of IT ($p < 0.01$). Mean IgG4 levels were at much lower initial levels before IT (0.004 $\mu\text{g/ml}$) but increased significantly to 0.11 $\mu\text{g/ml}$ at 1 year of IT and 1.19 $\mu\text{g/ml}$ at 2 years of IT ($p < 0.0001$). During early IT, RW IgG1 increased more rapidly than RW IgG4 and to higher levels. As the dose and duration of IT increased, RW IgG4 responses were significantly enhanced (Table I and Fig. 2). The ratios of RW IgG4/IgG1 increased during the course of IT from 0.004 before IT to 0.035 (ninefold)

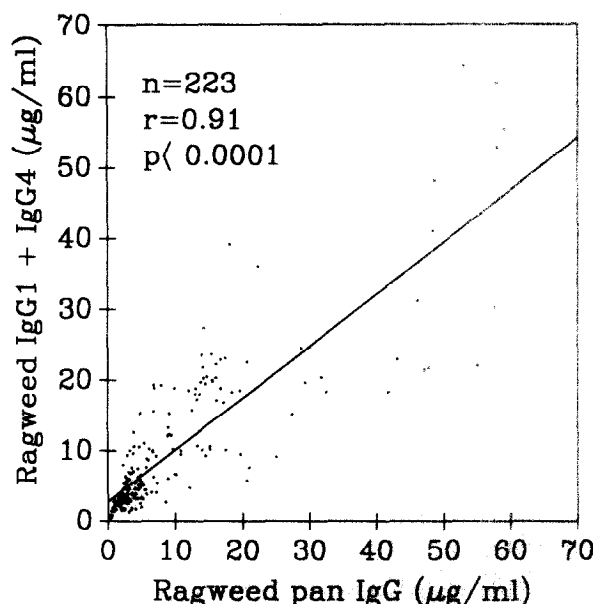


FIG. 1. Correlation between RW pan-IgG and sum of RW IgG1 plus RW IgG4. Solid line is least-squares, best-fits regression equation, $\text{RW pan-IgG (micrograms per milliliter)} = 0.018 \times \text{RW IgG1 (units per milliliter)} + 0.007 \times \text{RW IgG4 (units per milliliter)}$.

at 1 year of IT and to 0.259 (65-fold) at 2 years of IT. During years 5 to 8 of IT, the ratios of RW IgG4/IgG1 remained stable from 0.23 to 0.30, as illustrated in Fig. 3. For 2 years after IT was stopped, both RW IgG1 and RW IgG4 decreased progressively and significantly (Table II and Fig. 2), but the apparent average half-life of RW IgG4 (9 months) was significantly shorter than half-life of RW IgG1 (29 months) ($p < 0.001$) (Fig. 4). Despite the half-life differences, there was still a significant correlation in percent decline during 2 years between RW IgG1 and RW IgG4 ($r = 0.57$; $p < 0.02$) (data not presented). The ratios of RW IgG4/IgG1 decreased after IT was stopped from 0.29 at 1 month after IT to 0.11 at 1 year after IT and to 0.08 at 2 years after IT, whereas the ratios in the group that continued IT stayed almost unchanged (Fig. 3). There was no correlation between initial RW IgG1 levels or initial IgG4 levels and percent decline during 2 years in the group that stopped IT ($r = 0.12$ and 0.30 ; $p > 0.05$). These observations indicate that IgG1 is dominant in the early immune response of IT and disappears relatively slowly when IT is stopped. In contrast, IgG4 appears in significant quantities only later in the course of IT and disappears rapidly when IT is stopped.

RW IgE response

In Tables I and II and Fig. 5, the RW IgE response is presented during and after IT. Before IT, geometric

TABLE I. Serum RW IgG1, RW IgG4, and RW IgE antibody levels during 2 years of RW (geometric means)

	Course of IT					
	1985 pre-IT (N = 12)		1986 1-yr M-IT (N = 22)		1987 2-yr H-IT (N = 10)	
	Aug	Nov	Aug	Nov	Sept	Nov
RW IgG1						
μg/ml	1.13	0.95	3.14*	3.36*	4.77*	5.09*
% Of final	(22)	(19)	(62)	(66)	(94)	(100)
RW IgG4						
μg/ml	0.004	0.005	0.11†	0.18†	1.19†	1.32†
% Of final	(0)	(0)	(8)	(14)	(90)	(100)
RW IgE						
ng/ml	35	63‡	62	50‡	29	27

*Significantly different from pre-IT level; $p < 0.01$ (Student's unpaired t test).†Significantly different from pre-IT level; $p < 0.0001$ (Student's unpaired t test).‡Significantly different from preseasonal level; $p < 0.01$ (Student's paired t test).**TABLE II.** Geometric mean serum RW IgG1, RW IgG4, and RW IgE antibody levels during 2 years after RW IT was stopped

Antibody	In November 1985 Treatment group	1985		1986		1987	
		Aug	Nov	Aug	Nov	Sept	Nov
RW IgG1 (μg/ml)	IT stopped N = 16	7.44 (100)	6.88 (90)	4.88* (66)	4.78* (64)	3.96* (53)	3.91* (53)
	IT continued N = 15	5.23 (100)	5.03 (96)	5.52 (106)	5.06 (97)	4.63 (89)	4.88 (93)
RW IgG4 (μg/ml)	IT stopped N = 16	2.13 (100)	1.84 (86)	0.55* (26)	0.48* (23)	0.31* (15)	0.28* (13)
	IT continued N = 15	1.34 (100)	1.14 (85)	1.63 (122)	1.38 (103)	1.42 (106)	1.47 (110)
RW IgE (ng/ml)	IT stopped N = 16	29.0 (100)	33.5 (117)	21.9 (76)	45.1†‡ (155)	38.7 (133)	43.9† (151)
	IT continued N = 15	21.4 (100)	24.2 (113)	19.8 (92)	23.3 (108)	18.5 (87)	19.9 (93)

Percent of initial level in parentheses.

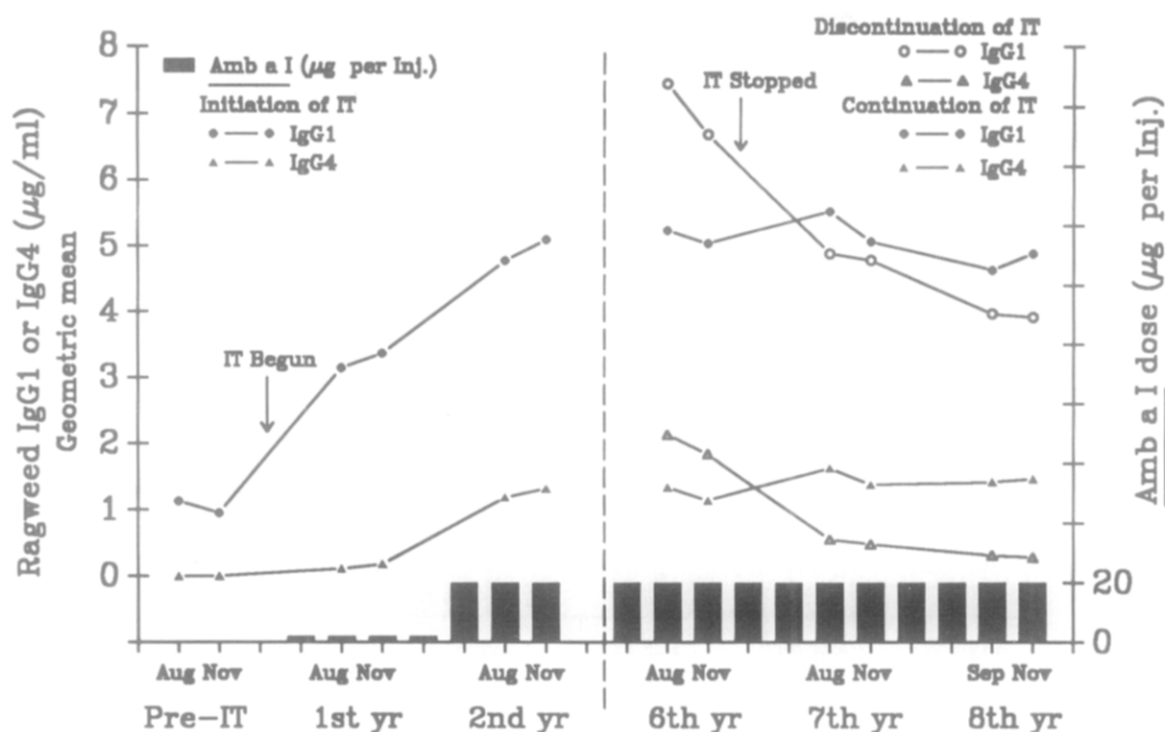
*Significantly different from initial level; $p < 0.0001$ (Student's paired t test).†Significantly different from the level of November 1985; $p < 0.05$ (Student's paired t test).‡Significantly different from preseasonal level; $p < 0.002$ (Student's paired t test).

mean RW IgE level rose 180% during the RW pollination season (August through November) from 35 ng/ml to 63 ng/ml ($p < 0.01$). This seasonal rise was ablated after IT from 1 year up to 8 years but returned after RW IT was stopped in 1986 (21.9 ng/ml before season to 45.1 ng/ml after season; $p < 0.002$). After 5 to 8 years of IT, mean RW IgE levels were unchanged across the RW season. The lack of a seasonal rise persisted in the group that continued IT ($p > 0.05$). Mean preseasonal RW IgE increased at 1-yr M-IT and then decreased at 2-yr H-IT, but there was no significant difference from

before IT RW IgE during the 2 years of treatment ($p > 0.05$) (Table I and Fig. 5). In the group that stopped IT, mean postseasonal RW IgE levels increased significantly from 33.5 ng/ml initially to 45.1 ng/ml 1 year later ($p < 0.02$) and remained at 43.9 ng/ml 2 years after IT was stopped ($p < 0.05$) (Table II and Fig. 5).

Intercorrelations

Before IT, RW IgG1 and RW IgG4 levels were not significantly correlated to RW IgE. During IT, especially when the dose of RW and duration of treatment



Course of immunotherapy

FIG. 2. RW IgG1 and RW IgG4 responses during ($N = 22$) and after IT (group stopped IT, $N = 16$; continued IT, $N = 15$). Both RW IgG1 and RW IgG4 significantly increased after IT ($p < 0.01$) and decreased after IT was stopped ($p < 0.0001$). RW IgG1 (circle) was dominant in early immune response of IT and disappeared relatively slowly when IT was stopped. In contrast, RW IgG4 (triangle) appeared in significant quantities only late in the course of IT and disappeared rapidly when IT was stopped.

increased RW IgG1 and RW IgG4 levels were increasingly correlated to RW IgE (Table III). After 2 years of IT, RW IgE levels were highly correlated to both RW IgG1 responses ($r = 0.94$; $p < 0.0001$) and RW IgG4 responses ($r = 0.81$; $p < 0.005$) (Fig. 6). Interestingly, RW IgG1 and RW IgG4 responses were not significantly correlated at any point (maximum, $r = 0.62$; $p = 0.056$) in this small group of patients (Table III).

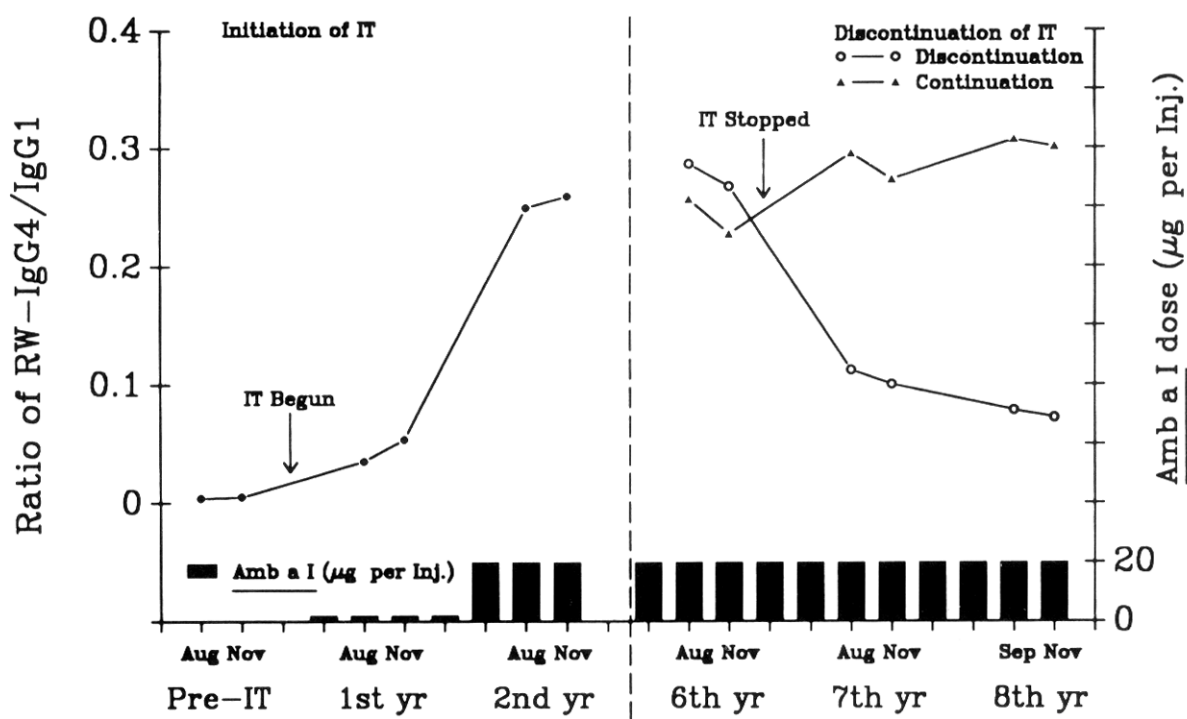
DISCUSSION

In our study, the 22 patients who received RW moderate-dose IT demonstrated reduced seasonal symptoms of allergic rhinitis with reduced early, late, and rechallenge reactions to RW challenge, as reported by Iliopoulos et al.²⁴ Another group of 16 patients who stopped RW IT had continued clinical improvement comparable to that of 15 patients who continued IT with RW (Norman et al.³⁴). In the present study we describe and discuss the antibody responses to the initiation and stopping of RW IT.

Quantitation of RW IgG1 and RW IgG4

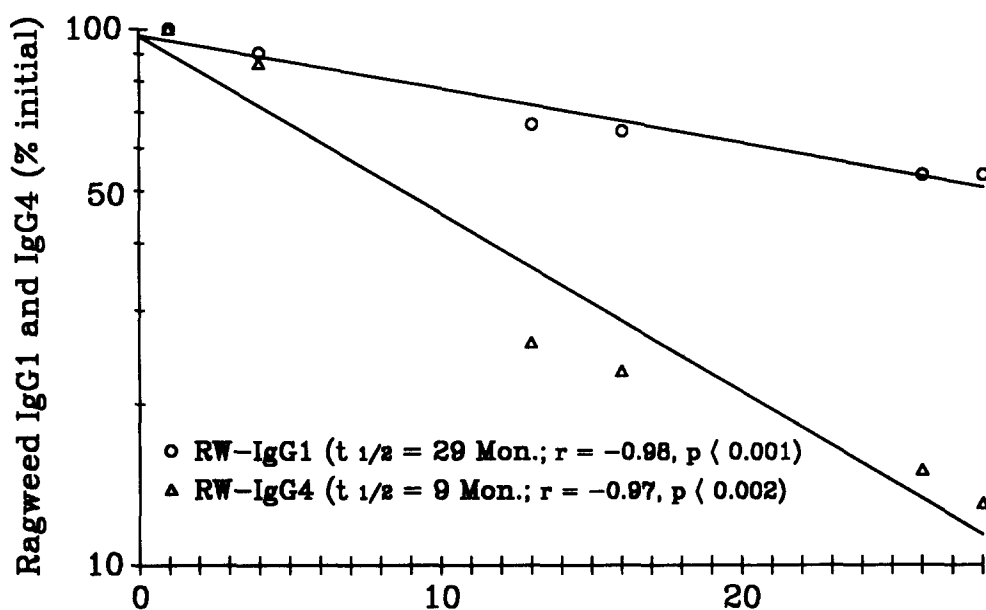
To date, no assays for IgG-subclass antibodies, thus far reported, have permitted measuring the absolute amount of IgG-subclass antibody. By use of an elution technique,^{27, 31} it should be possible to standardize solid-phase assays for measurement of IgG-subclass antibodies. However, we found no significant elution of IgG4 antibodies under conditions of alkaline pH previously successfully applied to IgG quantitation of yellow jacket-venom antibody.²⁷

Recent studies have illustrated that IgG-subclass responses during IT are largely restricted to subclasses 1 and 4.¹⁻⁹ We have previously estimated the absolute quantity of RW pan-IgG by a *Staphylococcus*. A solid-phase RIA elution technique.²⁷ Later, we developed an ELISA with monoclonal antihuman γ chain for quantitative estimation of RW pan-IgG.²⁸ These techniques made it possible to calibrate RW IgG1 and RW IgG4 responses in arbitrary units. In the present study we have estimated the absolute quantities of RW IgG1 and RW IgG4 by least-squares multiple regression analysis with the assumption that total IgG antibody



Course of immunotherapy

FIG. 3. Ratio of RW IgG4/IgG1, at the end of 2 years of IT, reached its peak, 0.25, which was very close to ratios in groups who had received IT more than 5 years (0.26 and 0.29). Ratio remained stable during continued IT and decreased after IT was stopped.



Month after discontinuation of IT

FIG. 4. Exponential plot of disappearance of serum RW IgG1 (○) and RW IgG4 (Δ) after RW IT was stopped. Slope estimates for RW IgG1 (29 months) and RW IgG4 (9 months) decay were significantly different ($p = 0.001$), indicating that half-life for RW IgG4 is shorter than that for RW IgG1.

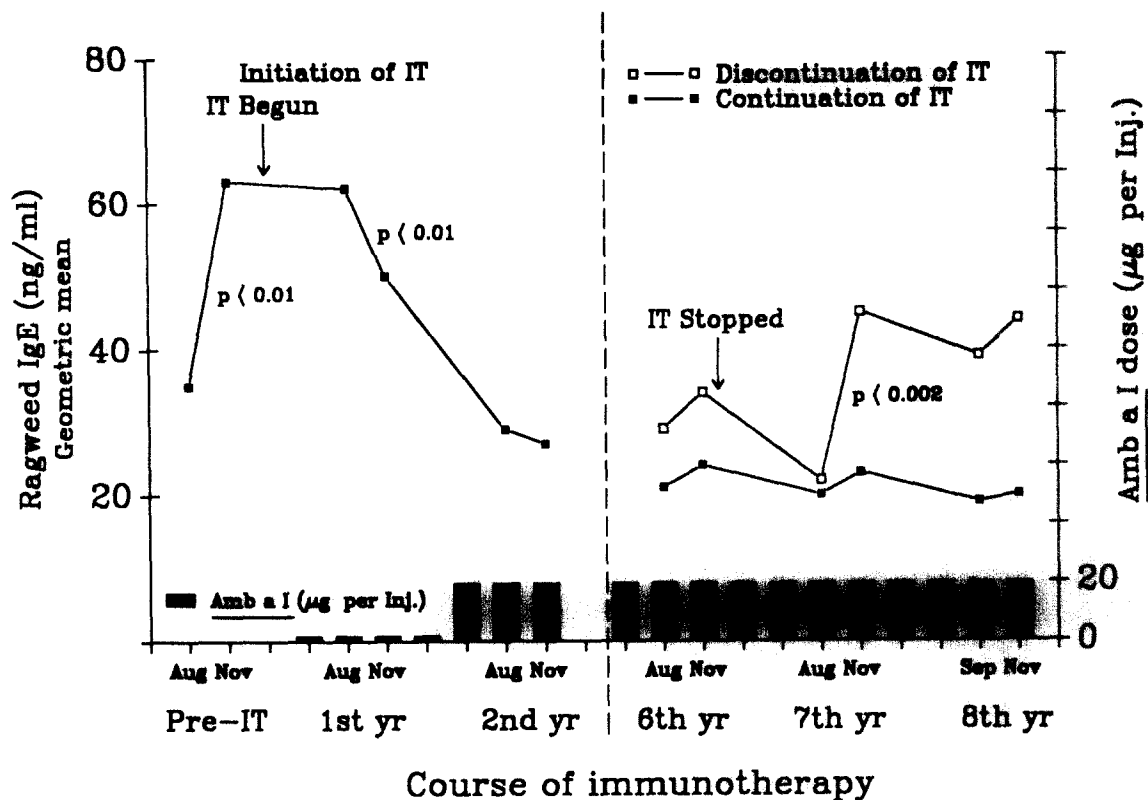


FIG. 5. RW IgE response during ($N = 22$) and after IT (group stopped IT, $N = 16$; continued IT, $N = 15$). Before IT, mean RW IgE rose 180% ($p < 0.01$) during the RW pollination season (August to November). During 2 years of IT and in a second group of subjects who received 5 to 8 years of IT, this seasonal rise was ablated (■) but returned 1 year after IT was stopped with a significant rise of RW IgE level ($p < 0.002$) (□). Arrows indicate the initiation of IT in February 1986 (left panel) and when IT was stopped for half of a different group in November 1985 (right panel).

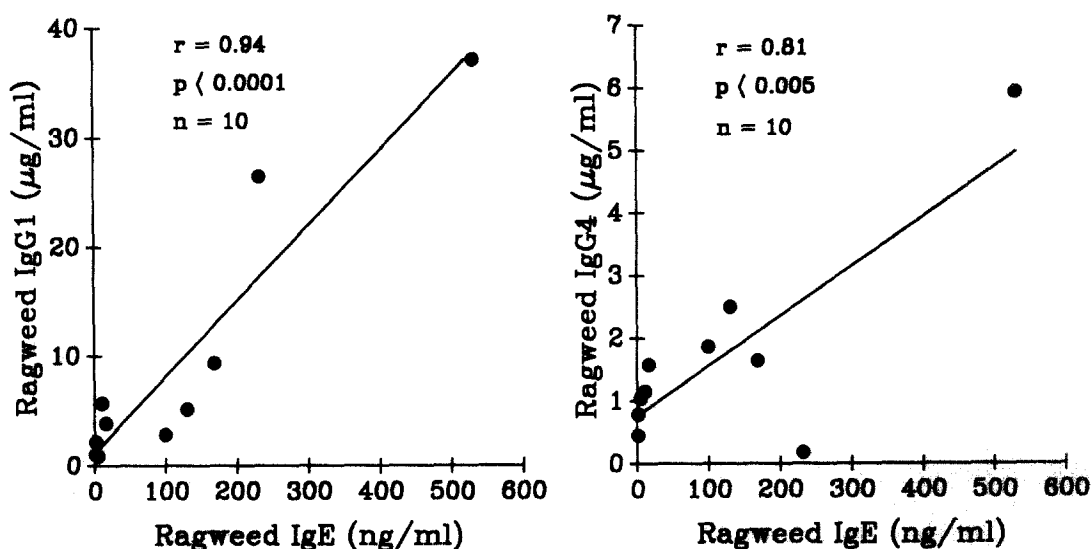


FIG. 6. Relationship between RW IgG1 (left panel) or RW IgG4 (right panel) and RW IgE after 2 years of RW IT.

TABLE III. Correlations and ratios among the serum RW IgG1, RW IgG4, and RW IgE antibody levels in patients during 2 years of RW IT

RW antibody	Pre-IT (N = 12)		1-yr m-IT (N = 22)		2-yr II-IT (N = 10)	
	Aug	Nov	Aug	Nov	Aug	Nov
IgG1/IgE						
<i>r</i>	-0.18	0.09	0.68	0.54	0.94	0.90
<i>p</i>	0.58	0.79	0.001	0.009	0.0001	0.001
Ratio	32	15	51	67	164	189
IgG4/IgE						
<i>r</i>	-0.19	-0.12	-0.06	-0.04	0.81	0.70
<i>p</i>	0.55	0.71	0.79	0.87	0.005	0.02
Ratio	0.11	0.08	1.77	3.60	41.0	48.9
IgG4/IgG1						
<i>r</i>	0.40	0.23	0.27	0.21	0.62	0.41
<i>p</i>	0.20	0.47	0.23	0.34	0.056	0.24
Ratio	0.004	0.005	0.035	0.054	0.249	0.259

is accounted for entirely by IgG1 and IgG4. The strong correlation between RW pan-IgG and the sum of RW IgG1 plus RW IgG4 ($r = 0.91$; $p < 0.0001$) suggests that this assumption is largely correct. This method overcomes numerous obstacles in quantitating IgG-subclass antibody³⁵ and may be applicable to other antigen specificities with restricted subclass responses.

RW IgG1 and RW IgG4 responses

With these ELISA assays we measured RW IgG1 and RW IgG4 responses during and after IT. Our data reveal that, early in the course of IT, IgG1 antibody responses were dominant; after 1-yr M-IT, the ratio between IgG4 and IgG1 antibody means was about 0.04. As the dose and duration of IT increased, IgG4 responses accelerated, but IgG1 remained dominant, as demonstrated by the maximum IgG4/IgG1 ratio of 0.25 (Fig. 3). This result largely agrees with the results of previous studies. An early IgG1 response and a late onset and continuously increasing IgG4 response were also observed in IT with timothy-grass pollen,⁴ house dust mite,^{36, 37} and mold.⁸ A shift in the IgG4/IgG1 antibody ratio, finally resulting in IgG4 dominance, was found in the case of beekeepers in whom the quantitation of IgG4 antibodies was achieved by Sepharose-coupled anti-IgG4.³⁸ In the present study, it is remarkable that in two independent study groups, the levels of RW IgG1 and RW IgG4 are comparable after 2 and 5 to 8 years of RW IT. The ratio of RW IgG4/IgG1 at the end of 2 years of IT reached its peak, 0.25, which was very close to the ratios in the groups who had received IT for more than 5 years (0.26 and 0.29) (Fig. 3). These data indicate that, during IT, IgG4 increases until it is about

20% of the IgG antibody response and then remains stable during long intervals of continued IT. These data also demonstrate a differential decay of IgG1 and IgG4 antibodies after IT is stopped. This finding partly agrees with a previous study by Djurup and Osterballe⁴ in which 1 year after IT was stopped with grass-pollen extract, 6/16 (38%) subjects still had significant levels of both IgG1 and IgG4; 7/16 (44%) had IgG1 only, and 3/16 (19%) had IgG4 only. These findings contrast with the study by Urbanek et al.⁶ in which IgG1 antivenom fell to pre-IT levels after 3 years of IT in contrast to IgG4 levels, which declined relatively little in the 2 years after termination of IT.

RW IgE response

These current data confirm several studies that long-term IT results in blunting of the expected IgE response to seasonal pollen exposure and gradually suppresses serum IgE antibody levels down to, but not much below, pretreatment levels.¹⁰⁻¹⁷ After IT was stopped, IgE response to seasonal pollen exposure returned completely the following years (Fig. 5). In the following season, the preseasonal serum samples were collected in September at the middle of RW season, by which time the seasonal rise had already occurred. Therefore, a statistically significant seasonal rise in RW IgE was not observed in 1987. As for IgG subclass antibodies, RW IgE levels at the end of 2 years of IT (27 ng/ml) were very close to the levels of comparable patients who had received 5 to 8 years of IT (~25 ng/ml). This finding strongly suggests that prolonged IT with standard methods does not result in progressive decline of IgE synthesis below pretreatment levels. However, mean postseasonal RW

IgE levels increased significantly at 1 year ($p < 0.02$) and 2 years ($p < 0.05$) after IT was stopped (Table II), indicating a rebound from the suppressive effects of IT.

Intercorrelation for RW antibodies

Our data demonstrated that intercorrelations among RW IgG1 or RW IgG4 and RW IgE became increasingly significant with escalating dose and duration of IT (Table III). This result is in agreement with the results of Djurup and Østerballe⁴ in which serum IgE, IgG1, and IgG4 antibodies were all mutually intercorrelated at the end of 116 weeks of IT with grass pollen. However, the degree of correlation between IgG and IgE levels during IT has varied in different studies. Gleich et al.¹¹ and Yunginger and Gleich³⁹ previously demonstrated positive correlations between IgG and IgE levels after a single season of high-dose RW IT ($N = 25$; $r = 0.73$; $p < 0.001$) and subsequent perennial IT averaging 6 years ($N = 63$; $r = 0.67$; $p < 0.01$). Creticos et al.,¹⁶ however, did not find a significant correlation of the maximum response between RW IgG1 and RW IgG4 or of the maximum increments in RW IgG and RW IgE. The strong association we found suggests that in IT the intensity of RW IgG1 and RW IgG4 responses are linked to that of RW IgE, despite low-dose mucosal presentation for IgE and high-dose parenteral immunization for IgG1 and IgG4 responses.

The possible role of IgG1, IgG4, and IgE during IT

One possible beneficial role of IgG1 and IgG4 antibodies formed during IT would be to neutralize allergens before they reach cell-bound IgE antibody, thereby serving as blocking antibodies in the traditional sense. Positive correlations between increased IgG1⁷ or IgG4^{37, 40} and clinical improvement during IT have been reported. The best clinical example of such IgG protection is in beekeepers, most of whom are protected by frequent stings from allergic reactions despite often considerable IgE antibody titers to honeybee venom; such patients have large quantities of predominantly IgG4 antibodies.³⁸ In contrast, negative correlations between IgG1 or IgG4 responses and clinical improvement were found in two Danish studies of grass and *Cladosporium* IT.^{4, 8} A good clinical prognosis significantly correlated with a low specific IgG4 titer in the study of dust mite IT by Etievant et al.⁴¹ In general, prolonged and persistent antigen stimulation appears to favor IgG4 antibody production not only in the later phases of IT but also in beekeepers with more than 3 years experience,² patients with chronic parasitic infestations,⁴² patients with Hashi-

moto's thyroiditis (autoantibodies to thyroglobulin),⁴³ and hemophiliac patients (to factor VIII).⁴⁴ By contrast, IgG1 antibodies are most prominent in the following: (1) in early responses to allergen IT, (2) individuals with limited antigen stimulation, such as prophylactic immunization with tetanus toxoid or incidental bee stings,² and (3) patients receiving insulin injections.⁹ Experiments in animals^{45, 46} have demonstrated that cytophilic IgG subclasses may participate in feedback inhibition of IgE antibody production, and some evidence has been provided that specific IgG may down regulate IgE antibody production in allergic patients.⁴⁷ A more recent study demonstrated that T cells generated during IT can be activated by RW antigen in vitro to suppress RW IgE production, whereas RW antigen enhance RW IgG production.⁴⁸ Thus, biologic efforts of allergen-specific IgG1 and IgG4, formed during IT, are potentially numerous and complex. Whether any of these effects lead to symptom relief remain speculative, since the IgG antibody response, which invariably accompanies successful IT, may be only an epiphenomenon and causally unrelated to the mechanism of clinical desensitization. The relative roles of IgG1 vis a vis IgG4 have been even more difficult to discern. The quantitative methods we have developed in the current study may prove useful in further attempts to delineate the functional significance of IgG1- and IgG4-antibody responses during allergen IT.

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Immunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety

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Venom immunotherapy (VIT) for Hymenoptera allergy is accepted as safe and effective. However, widely varying success rates and frequencies of side effects are reported. Differences between various Hymenoptera species could account for these diverging results. We therefore analyzed 205 patients with a history of systemic allergic reactions to either honeybee (148 patients) or yellow jacket stings (57 patients) during VIT. All patients had a positive skin test to the respective venom before VIT, were monitored for side effects of VIT, and submitted to a sting challenge while they were receiving VIT. Patients with honeybee-venom allergy had a higher sensitivity in both skin tests ($p < 0.05$) and RAST ($p < 0.001$) than patients with yellow jacket-venom allergy. They developed systemic side effects to VIT injections significantly more often (41% versus 25%; $p < 0.01$) and also reacted more frequently to the sting challenge (23% versus 9%; $p < 0.01$) than patients with yellow jacket-venom allergy. We conclude that results obtained from studies on the allergy to one Hymenoptera venom cannot be extrapolated to allergies to other Hymenoptera venoms. (J ALLERGY CLIN IMMUNOL 1992;89:529-35.)

Key words: Hymenoptera-sting allergy, honeybee venom, yellow jacket venom, venom immunotherapy

VIT is accepted as a safe and effective treatment for patients with allergic SRs after Hymenoptera stings.¹⁻³ However, widely varying success rates are reported from different centers. According to a CH

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Abbreviations used

VIT:	Venom immunotherapy
SE:	Systemic allergic side effects
SSE:	Subjective SE
OSE:	Objective SE
HB:	Honeybee
YJ:	Yellow jacket (<i>Vespa</i> sp)
HBV:	Honeybee venom
YJV:	Yellow jacket venom
EPC:	End point concentration
CH:	Sting challenge
SR:	Systemic reaction