

A subclass IgG4-specific antigen-binding radioimmunoassay (RIA): Comparison between IgG and IgG4 antibodies to food and inhaled antigens in adult atopic dermatitis after desensitization treatment and during development of antibody responses in children

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A liquid-phase, antigen-binding radioimmunoassay measuring subclass IgG4 antibody (ab) to allergens has been developed. This assay, which uses monoclonal anti-IgG4 to bind IgG4, allows direct comparison of class (IgG)- and subclass (IgG4)-specific ab levels. These assays used radiolabeled purified allergens, Der p I (Ag P₁) from the dust mite Dermatophagoides pteronyssinus, Lol p I (Rye 1), from ryegrass pollen, hen's egg ovalbumin, and β -lactoglobulin from cow's milk. We have investigated IgG4 abs in several clinical situations. The results confirm that IgG ab responses to both inhalants and food proteins unequivocally include IgG4 ab. On average, the proportion of IgG4 ab to these antigens is far higher than the contribution of IgG4 to total IgG. In patients with adult atopic dermatitis, levels of both class and subclass ab were higher than in control subjects; however, the ratio of IgG4:IgG varied widely in patients and control subjects. During desensitization treatment of patients with perennial rhinitis, levels of IgG4 ab to Der p I increased sharply, but there were also increases in the total IgG ab responses so that the percentage contribution of IgG4 was only moderately increased (mean values: before, 29%; after, 36%). In a prospective study of children from atopic families, IgG4 abs to food proteins were detectable as early as 3 months. IgG abs to hen's egg ovalbumin and β -lactoglobulin from cow's milk increased to a maximum at 3 years and declined by 5 years. However, specific IgG4 as a percentage of specific IgG increased progressively from a mean value of ~15% at 6 months to ~50% at 5 years of age. Overall, the results do not support a role for IgG4 in immediate skin reactivity as the sole blocking ab or in the production of allergic symptoms. However, given the known properties of the IgG4 subclass, changes in IgG4 abs to food allergens could well alter the impact of these proteins on young children. (J ALLERGY CLIN IMMUNOL 1987;80:622-30.)

The role of IgG4 subclass abs in the pathogenesis of allergic diseases has given rise to intense speculation. This subclass, which normally comprises only 4% of the total serum IgG, has been demonstrated to

increase in atopic dermatitis as well as in a variety of other allergic diseases.¹⁻³ In general, specific ab responses to protein antigens are predominantly IgG1. However, specific ab responses, including IgG4, have been reported against a number of foods, pollens, venoms, and other protein antigens.⁴⁻⁷ The report that IgG4 can sensitize peripheral blood basophils to release histamine and the description of non-IgE short-term sensitizing activity in the IgG ab fraction has suggested an effector role for this subclass.^{8,9} However, in most cases in which individuals have specific IgG4 ab and no detectable IgE ab, they are found to be skin test negative and asymptomatic.^{5,6} In addition, the study that desensitization therapy can result in increased levels of IgG4 ab has led to speculation that the subclass can have a role as blocking ab.¹⁰

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Abbreviations used

ab:	Antibody
Der p I:	Der p I from <i>Dermatophagoides pteronyssinus</i> (previously antigen P ₁)
BA:	Binding activity
BBS:	Borate-buffered saline, pH 8.0
β-lac:	β-lactoglobulin from cow's milk
OA:	Hen's egg ovalbumin
Lol p I:	Group I protein from ryegrass pollen (previously Rye I)
RIA:	Radioimmunoassay

Previously announced measurements of subclass-specific ab have predominantly used solid-phase antigen and have not measured overall IgG ab with a directly comparable technique. Fluid-phase antigen-binding assays with radiolabeled purified antigens and with precipitation of class and subclass with anti-globulin reagents have several advantages.¹¹ In these assays the solid phase has no influence on binding of antigen. Also, the quantity of immunoglobulin in the serum generally has no effect on the nonspecific background. We report here the development of antigen- and allergen-binding RIAs in which IgG4 abs are selectively bound by monoclonal anti-IgG4 and, subsequently, precipitated by antiserum to the monoclonal reagent. This assay has been used to compare the relationship of IgG4 ab to overall IgG ab with two food antigens, OA and β-lac, and two inhalant allergens, Lol p I and Der p I (or antigen P₁). These assays have been used to measure serum IgG4 ab in three situations, atopic dermatitis, desensitization treatment, and the onset of ab responses in early childhood.

MATERIAL AND METHODS**Patients**

Serum samples analyzed in this study were all obtained from patients involved in separate studies that have been reported previously.

Adult patients with atopic dermatitis (N = 21) were randomly selected for study from a larger group.^{12, 13} Skin prick testing to a range of common allergens (Bencard Ltd., Betchworth, U. K.) was performed, and positive responses, as determined by a wheal diameter 3 mm or more greater than the negative control site at 15 minutes, were observed with egg (n = 11), milk (n = 3), house dust mite (n = 21), and grass pollen (n = 18). All patients had been diagnosed on the basis of a characteristic pruritic skin rash, with extensive involvement of moderate to severe intensity. The mean age was 29 years (range 15 to 60 years), and all patients had suffered from the disease for more than 10 years. The control group comprised 10 nonatopic subjects with negative skin prick tests, no history of skin disease, and a mean age of 30 years (range 25 to 53 years).

As previously reported,¹⁴ adult Chinese patients with

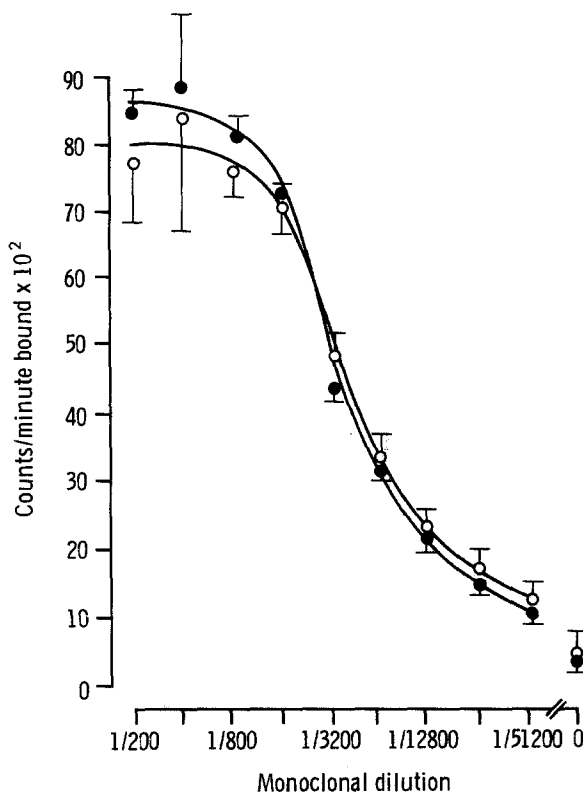


FIG. 1. Duplicate dilutions (100 μ l) of the monoclonal anti-IgG4 clone RJ4 ($\circ$) or a mixture of clones RJ4 and GB12 ($\bullet$) in 1/50 normal mouse serum were added to an atopic serum containing high-titer IgG anti-OA that had been incubated for 3 hours with radiolabeled OA. After an additional 3 hours of incubation, rabbit antimouse IgG was added to precipitate the soluble complexes formed. Bars indicate range for duplicate values at each point.

more than a 3-year history of perennial rhinitis and positive skin and nasal challenge tests to *D. pteronyssinus* extract had been allocated to two treatment groups. One group (N = 20) received graded doses of aqueous house dust-mite extract (Bencard), 0.0004% to 0.2%, whereas the placebo-treated group (N = 20) received saline injections. The patients had been followed during a period of 18 months with initial weekly injections followed by monthly maintenance doses for more than 1 year. Serum samples were obtained before treatment and 6 months after starting maintenance therapy. The sera of 10 subjects randomly selected from each treatment group were analyzed.

Ninety-two children, having one or more atopic parents, were followed prospectively from 3 months to 5 years, undergoing regular assessment, skin prick testing, and serum sampling.¹⁵ For the present study, representative groups were selected on the following basis: (1) known IgG ab profiles, (2) IgE ab responses, (3) skin test reactivity, and (4) sample availability for different ages.

Antigen-binding RIA for class-specific ab

Antigen-binding assays were used to quantify specific IgG and IgE ab.^{11, 13, 16} Radiolabeled antigen (3 to 7 ng) was

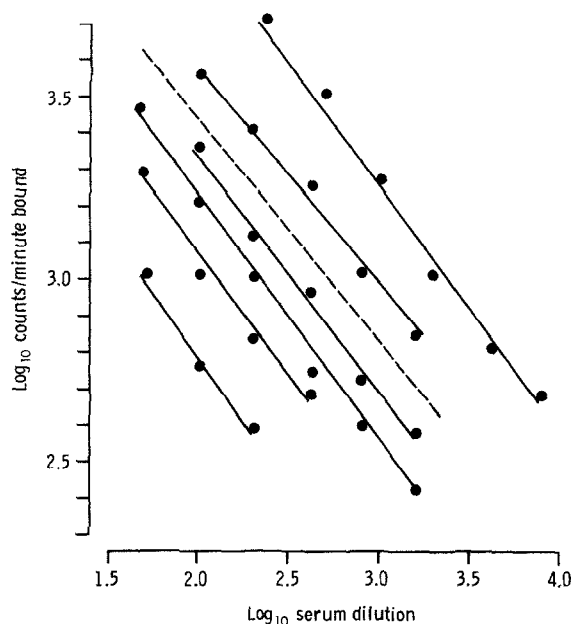


FIG. 2. Regression analysis of IgG4 dilution curves of six sera with IgG4 BA to OA. Increasing dilutions of the sera were incubated in duplicate with 3 ng of ^{125}I -labeled OA and a 1/100 dilution of monoclonal anti-IgG4 in 1/50 normal mouse serum for 4 hours before precipitation with rabbit antimouse IgG. A standard IgG ab control curve for OA was also produced and is superimposed on the figure for comparison. Background binding was lower for the subclass assay. To enable reading of results from the IgG ab control curve for the subclass assay, the difference in the mean background counts for four negative sera in both assays was added to the subclass assay counts before estimation of BA. The lines of best fit for each curve (—) and a standard IgG OA control curve (---) are illustrated for $\log_{10}$ transformed data.

incubated at room temperature with 0.1 ml of a 1/12.5 to 1/100 dilution of serum diluted in 0.3% human serum albumin in BBS, pH 8.0, for more than 3 to 4 hours. The quantity of radiolabeled antigen is constant for each assay and was in the range of 3 to 7 ng. Class-specific ab was precipitated overnight (4°C) with goat antihuman IgG or IgE. After washing (three times) in BBS, the residual radioactivity in the precipitate was estimated in a gamma counter (NE1600, Nuclear Enterprises, Edinburgh, U. K.). Results for each assay were expressed in arbitrary units of BA with reference to doubling dilutions of a standard serum for each antigen.

Antigen-binding RIA for subclass-specific ab

To quantify IgG4 subclass responses to purified allergens, a subclass-specific antigen binding assay was developed. In this assay, mouse monoclonal antihuman IgG4 subclass reagents specific for the Fc fragment of IgG4 (clone numbers RJ4 and GB12, a kind gift from Dr. R. Jefferis, University of Birmingham), supplied as ascites fluid, were used. As in the class-specific ab assay, radiolabeled antigen (100 μl

containing 3 to 7 ng; quantity varies with different antigens but is constant for a given antigen) was added to 100 μl of a serum dilution and followed by 100 μl of monoclonal anti-IgG4 diluted in 1/100 normal mouse serum. This mixture was incubated at room temperature for 4 hours. Subsequently, rabbit antimouse IgG antiserum sufficient to precipitate all the mouse IgG present was added, followed by overnight incubation at 4°C . The precipitate formed was washed three times with BBS, and the remaining radioactivity was counted in a gamma counter. To ensure precipitation of IgG4 ab antigen complexes alone, antigen must remain in excess, and this was achieved by use of dilutions of sera that bound <20% of the total precipitable counts added.¹⁵ The counts bound were converted to units of BA by reference to a total IgG ab standard curve run in parallel.

RAST

Egg and milk antigens were coupled to filter paper disks (Whatman No. 541) previously activated with cyanogen bromide.¹⁷ A modified RAST method (as previously described) was used to measure IgE ab to egg and milk antigens.¹³ Briefly, antigen-coated disks were incubated overnight at room temperature in patients' sera and were diluted 1:10 in horse serum. After extensive washing in RAST buffer (BBS and 0.5% Tween 20), the disks were incubated overnight at room temperature with ^{125}I -labeled anti-IgE and again were diluted in horse serum. After further washing in RAST buffer, the remaining radioactivity was determined with a gamma counter. The results were compared with the mean binding obtained from five cord sera (negative control) or a (positive) control curve made with serial dilutions of a strongly positive serum.

Total IgE levels were measured with a double ab inhibition RIA.¹⁸

Statistics

The Spearman rank test was used to assess the significance of correlations between antigen-specific IgG4, IgG, and IgE ab. The Mann-Whitney U test was used to compare statistical differences between the mean levels of IgG4, IgG, and IgE ab for the various groups.

All procedures involving human subjects had been approved by the appropriate hospital ethical committees.

RESULTS

IgG4 subclass assay

Serial dilution of the monoclonal reagent was used to identify the quantity that was required to ensure precipitation of all the IgG4 present in a serum sample (Fig. 1). The quantity of anti-IgG4 ab that was necessary was estimated by the incubation of sera with known total IgG4 and demonstrable IgG4 ab in the presence of decreasing dilutions of monoclonal reagent.¹⁹ It was assumed that the affinity of the binding of the monoclonal reagent to antigen-complexed IgG4 was representative of the binding that occurs to all

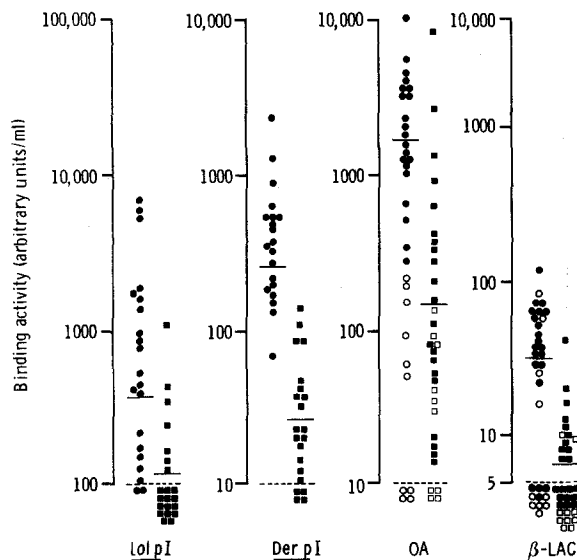


FIG. 3. IgG (●) and IgG4 (■) BA in 21 patients with atopic dermatitis (closed symbols) and in 10 control subjects (open symbols) to *Lol p I*, *Der p I*, OA, and β -lac. The minimum level of sensitivity for each antigen-specific assay is illustrated by the dashed line (---); the geometric mean levels for each antigen are illustrated by the bar (—).

IgG4 present. No increase in precipitation was evident when a combination of two anti-IgG4 monoclonal reagents was used (Fig. 1); therefore, a single monoclonal reagent was routinely used. Estimates for three sera of the quantity of IgG4 precipitated by duplicate dilutions of monoclonal reagents (range 1/100 to 1/12800) indicate that a 1/100 dilution was sufficient to precipitate $\sim 16 \mu\text{g}$ of IgG4. Subsequently 1/100 of the monoclonal antibody was used, and all sera were assayed at a dilution that ensured antibody excess, that is, 1/25 or greater.

The dilution curves for IgG and IgG4 ab for a number of sera appeared to be parallel with either OA or *Der p I*. This interpretation was confirmed by regression analysis (Fig. 2). We therefore used an IgG ab standard curve for quantitation of both IgG and IgG4 BA. To assess the time required for monoclonal ab binding to occur, preformed antigen-IgG4 ab complexes were incubated with monoclonal anti-IgG4 for time periods from 10 minutes to 5 hours. Maximal binding occurred within 10 minutes (data not presented). In addition, it was apparent that separate (3 hours) incubation steps for antigen and monoclonal reagent, respectively, were not necessary, since very similar BA was evident when the monoclonal reagent and the antigen were added to serum at the same time. This observation allowed us to use a single

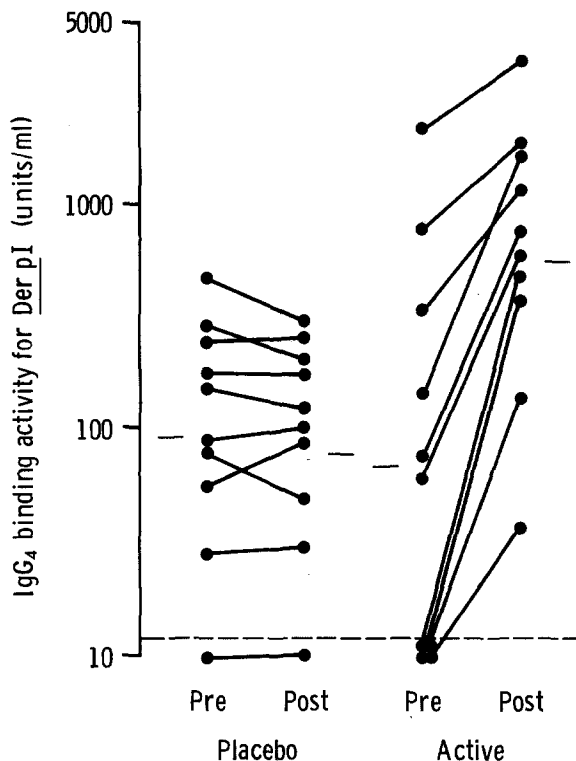


FIG. 4. IgG4 BA to *Der p I* in 20 patients with perennial rhinitis receiving active or placebo desensitization injections. Levels before (*pre*) and after (*post*) treatment are illustrated with the geometric mean values for each group (—). The dashed line (---) indicates the minimum sensitivity of each assay.

incubation step of 4 hours before the addition of the precipitating rabbit antiserum.

Subclass IgG4 ab to ingested and inhaled allergens in adult patients with atopic dermatitis

All adult patients with atopic dermatitis tested ($N = 21$) had both IgG and IgG4 abs specific for OA (Fig. 3). The subclass ab in these subjects comprised 20% (range 1% to 94%) of the total OA-specific IgG. We have previously confirmed that some nonatopic adult subjects have IgG ab to OA.¹³ The present study demonstrates that such individuals can also have IgG4 ab, and in the six nonatopic subjects (of 10) who possessed egg-specific IgG ab, IgG4 subclass ab comprised 61% (range 16% to 87%) of the specific IgG. The mean levels of both class- and subclass-specific IgG ab were significantly greater in the patients with atopic dermatitis than in nonatopic control subjects (mean IgG = 2089 versus 41 BA units per milliliter; mean IgG4 = 199 versus 30 BA units per milliliter) ($p = <0.012$). Eleven of the 21 patients had de-

TABLE I. Contribution of IgG4 ab to the IgG ab response to *Der p I* during immunotherapy with mite extract

	Placebo*		Active*	
	Pre	Post	Pre	Post
% IgG ab†	33 (7-64)	29 (10-65)	29 (13-47)	36 (12-67)

*Calculated for those sera with both IgG and IgG4 BA before desensitization; placebo, n = 9; active, n = 6.

†Arithmetic mean levels for each group. Values in parenthesis indicate the range of results observed. Percentage contribution of IgG4 ab

$$\text{to total IgG ab response} = \frac{\text{IgG4 BA/ml in serum}}{\text{IgG BA/ml in serum}} \times 100$$

Prevalues and postvalues were not significantly different with the Student's t test for paired samples for either group.

monstrable IgG4 subclass ab to β -lac, whereas 17 patients were demonstrated to have IgG ab (Fig. 3). The prevalence of both IgG and IgG4 ab to this antigen was higher in atopic subjects than in the nonatopic group.

As the percentage contribution of IgG4 to total IgG ab levels varied widely in subjects with atopic dermatitis for both egg (1% to 94%) and milk (7% to 62%), a possible association with skin test positivity was sought. Egg prick test positive subjects (N = 11) had a mean OA-specific IgG ab level of 2513 BA units per milliliter; the comparable figure was 1807 BA units per milliliter in prick test negative subjects (N = 8). (Skin test information was not available in two individuals). The mean level of OA-specific IgG4 in the prick test positive subjects was 190 units of BA per milliliter (mean contribution to IgG antibody = 16% (range 1% to 60%) and 236 units of BA per milliliter in the prick test negative group (mean contribution to IgG antibody = 24%; range 1% to 94%). Furthermore, the relationship between IgG4 and IgE specific ab levels to egg/OA in those subjects positive for IgE antibody (N = 11) was weak ($r = 0.202$; $p = >0.1$). Only three individuals were prick test positive to milk protein, and the levels of IgG4 abs in these patients were not different from the whole group. None of those subjects had demonstrable IgE antibody to milk protein by RAST. Overall the results do not suggest that IgG4 abs play any role in skin reactivity.

Only 7/19 adult subjects with atopic dermatitis and with IgG ab specific for *Lol p I* also had IgG4 subclass ab. Seventeen of 20 subjects with IgG-specific ab to *Der p I* had the subclass ab (Fig. 3). There was a good quantitative correlation between the levels of IgG and IgG4 ab: for *Der p I*, $r = 0.65$ ($p = <0.01$), and for *Lol p I*, $r = 0.57$ ($p = <0.02$). In subjects with both class and subclass ab to inhalant antigens, specific IgG4 made a smaller percentage contribution to the total IgG ab than was the case with food an-

tigens. *Lol p I* was 13% (range 3% to 25%) and *Der p I* was 11% (range 1% to 25%). In ab positive subjects, the relationship between IgG4 and IgE specific ab was very weak for *Der p I* ($r = 0.364$; $p = >0.05$). Despite the small numbers, there was a significant relationship between IgG4 and IgE in the case of *Lol p I* ($r = 0.891$; $p = 0.01$). There was no association between total serum IgE level and specific IgG4 levels for any of the allergens studied ($p = >0.05$).

House dust-mite antigen desensitization and IgG4 specific ab

Fifteen of 20 subjects with house dust-mite sensitivity and perennial rhinitis had demonstrable IgG4 ab to *Der p I* before desensitization (Fig. 4). Desensitization resulted in a significant increase in the subclass ab level in all 10 subjects undergoing this procedure ($p = <0.03$), whereas no rise in ab levels was apparent in 10 subjects receiving placebo injections. In the actively treated group, there were four patients who produced mite-specific IgG4 abs during treatment despite the ab initially being undetectable (Fig. 4). The percentage contribution of IgG4-specific ab to the total specific IgG ab was not significantly changed in the six patients who had IgG4 before treatment ($p = >0.05$) (Table I). However, excluding the four patients who had negative IgG4 before treatment reduced the chances of observing a significant result. Thus, our results are not contradictory to previous results, suggesting that repeated antigen stimulation can selectively stimulate IgG4 abs.

The development of food-specific IgG4 ab

In a previous study in which 92 children were prospectively followed during the first five years of life, we found that almost all the children had IgG ab to OA and β -lac within the first 12 months.¹³ Twelve children who had representative IgG ab profiles to

TABLE II. The development of IgG and IgG4 responses to food allergens during the first 5 years of life

Age (months)	Ab to β -lac* (BA/ml)				Ab to OA* (BA/ml)			
	Whole group	Subjects with IgG4 BA		Mean $\pm$ %	Whole group	Subjects with IgG4 BA		Mean $\pm$ %
		IgG (N)	IgG4 (N)			IgG (N)	IgG4 (N)	
3	114 (10)	325 (3)	35 (3)	11	735 (3)	1857 (2)	323 (2)	18
6	368 (10)	1033 (4)	80 (4)	8	361 (10)	373 (4)	60 (4)	23
12	599 (7)	772 (6)	107 (6)	16	2046 (9)	5132 (6)	580 (6)	19
36	573 (9)	573 (9)	127 (9)	38	14460 (10)	14460 (10)	4750 (10)	36
60	181 (7)	231 (5)	99 (5)	50	5933 (10)	5933 (10)	2555 (10)	52

*Geometric mean levels, arbitrary BA units per milliliter. Figures in parenthesis indicate number with demonstrable binding activity.

†Mean percentage of IgG4 in the IgG ab response calculated for subjects with measurable IgG4.

these antigens were assessed for the presence of IgG4 subclass ab. During the 5-year period, IgG4 subclass ab and IgG ab to both antigens increased and then decreased (Table II). However, during these 5 years, the percentage contribution made by IgG4 ab to the IgG ab level increased progressively (egg, 18% to 52%; milk, 11% to 50%).

The relationship between IgG4 and IgG was also assessed in relation to skin tests or IgE ab results to egg in 39 3-year-old children. Those with skin and/or IgE positivity had very high levels of IgG4 ab to OA (Table III). This subclass in the positive children made the major contribution to total egg-specific IgG ab (55% and 62%, respectively, Table III).

DISCUSSION

The present results confirm that humoral responses to allergens involve the production of IgG4 ab, the quantity of which is definitely out of proportion to the normal quantity of IgG4 in the serum.⁵ This contrasts with the IgG response to many other protein antigens that predominantly involve the production of IgG1 and IgG3 subclasses.³ In keeping with previous results, our results suggest that the production of IgG4 is influenced by antigen dosage, route, and chronicity of exposure, as well as age and the atopic status of the individual.

Previously, measurement of IgG subclass ab has been reported with the use of a range of techniques.^{2, 20, 21} As different enzyme-labeled or radio-

labeled antiglobulin reagents were used in those assays, direct comparison of total specific and subclass-specific ab levels was difficult. In the liquid-phase antigen-binding RIA, quantitation is dependent on the precipitated labeled antigen and is far less dependent on the antiglobulin reagents used. Therefore, a major advantage of this assay is that a direct comparison between levels can be made. The subclass assay outlined is a modification of previously described antigen-binding assays and uses the same concentrations of purified antigen and similar assay conditions. The assay is dependent on the proven specificity of the anti-IgG4 reagent used. Our results demonstrate that a single monoclonal ab (clone RJ4) was sufficient to precipitate all the IgG4 ab. This is in keeping with the study that a single monoclonal reagent was comparable to polyclonal anti-IgG4 in a RAST subclass assay.²² Based on studies of IgG4 ab cross-linking solid-phase antigen and fluid-phase radiolabeled antigen, it has been suggested that IgG4 ab may be functionally monovalent.⁵ In the antigen-binding assays, it is assumed that at antigen excess, ab is present primarily as ab (Ag)₂, that is, bivalent. Monovalency would result in underestimation of the level of subclass ab that is present, and where a significant percentage of class-specific IgG ab is subclass IgG4, underestimation of the class-specific IgG ab would result also. Without purified IgG4 ab, it would be difficult to resolve this issue of the functional valency of IgG4 ab.⁵

The significance of IgG4 in disease expression re-

TABLE III. The relationship between IgG4 ab to OA and the presence of IgE ab or skin tests in 39 children aged 3 years

BA (units/ml)	Skin test*		IgE ab†	
	+ ve (N = 11)	- ve (N = 28)	+ ve (N = 12)	- ve (N = 27)
IgG‡	22222 (50000-4500)	4889 (47000-37)	31058 (50000-9680)	4882 (22000-37)
IgG4‡	10683 (43200-1140)	1115 (24400-10)	18653 (43200-5200)	1030 (24400-10)
%IgG4§	55 (11-85)	37 (1-79)	62 (36-88)	33 (1-79)

+ ve = positive; - ve = negative.

*Among the skin test negative children, there was no correlation between IgG4 abs to OA and the presence or absence of eczema.

†IgE ab estimated by RAST.

‡Geometric mean levels. Values in parenthesis indicate range of results observed.

§The arithmetic mean percentage contribution of IgG4 to the IgG ab response. Values in parenthesis indicate range of results.

mains unclear. Assessment of levels in a variety of clinical situations fails to indicate a particular role for this subclass ab. We have demonstrated that adult patients with atopic dermatitis possess OA-specific IgG4 ab. However, it is known that such individuals possess IgG ab against this protein,¹³ and direct comparison demonstrates that IgG4 contributes a variable percentage to this total. There are differences between the responses to other food proteins in these same patients, and also differences between atopic and non-atopic subjects for the same antigen. The case remains, however, that the percentage contribution made by subclass IgG4-specific ab to the total level of specific IgG ab can be widely different in both allergic and nonallergic individuals. Differences in prevalence and percentage contribution between foods and inhaled allergens can be demonstrated, but differences between a seasonal and a perennial inhaled allergen were also found. In addition, the age of the subjects studied influenced the results. With OA, very high levels of IgG and IgG4-specific ab developed within the first 3 years, and the IgG4 subclass ab increased to contribute a large percentage to the overall IgG ab level. By year 5, however, levels of both abs were falling toward levels observed in adult subjects with atopic dermatitis. It is obvious that knowledge of the presence or level of IgG4 ab alone cannot be used to support a pathogenic role for this ab in atopic dermatitis.

Food and inhalant allergen-specific IgE ab is very unusual in human sera in the absence of an IgG ab response.^{11-13, 23} On the basis of previous studies, the relationship between specific IgE and IgG4 ab responses is difficult to interpret.^{22, 24, 25} Our result demonstrates a strong quantitative relationship in young

children between egg-specific IgG4 ab levels and both IgE ab and skin test responsiveness. This relationship was not present in adults with atopic dermatitis either for egg allergen or the perennial house dust-mite allergen in which all patients with specific IgG4 also had IgE ab. The results with the seasonal allergen *Lol p I* demonstrated a strong relationship in that minority of sensitive individuals who had both ab classes. This result was not explained by prior desensitization therapy. It is clear therefore that relationships between IgG4 and IgE ab can only be demonstrated in selected circumstances.²⁴

As the prevalence of IgG4 ab in patients with atopic dermatitis was higher for perennial (17/21) than for seasonal allergens (7/21), chronicity of exposure may be an important factor in the development of IgG4 responses.^{5, 25} The findings in perennial rhinitis infer that such effects are not limited to atopic dermatitis. Supporting this concept, repeated exposure, as occurs in desensitization, results in a marked increase in IgG4 levels. That cumulative exposure rather than chronicity may be the major determining factor is suggested by the profile of responses observed to food antigens in early life; specific IgG4 ab to foods was demonstrable almost as soon as IgG ab. By contrast, the development of IgG4 responses to inhalant allergens may be delayed by up to 2 years after IgG (data not presented), perhaps reflecting the much lower exposure levels to mite and grass-pollen allergens.

Although our experiments do not exclude a role for IgG4 ab in the pathogenesis of allergic disease, we have not demonstrated that the presence of IgG4 ab is correlated with any particular clinical situation. In this respect it would be of interest to know whether non-IgE-mediated food hypersensitivity, particularly

in early life, for example, Heiner syndrome²⁶ or gastroenteropathy,²⁷ could be associated with deficient or delayed development of IgG4 abs to food proteins. It is well established that IgG4 does not fix complement and is a poor opsonin.²⁸ This subclass also can block the activity of human IgE in a monkey passive cutaneous anaphylaxis response.²⁹ The demonstrated increase in IgG4 ab levels in patients desensitized to both pollens and venoms has been interpreted as supporting a protective role for this subclass ab.^{21,30} However, our results demonstrate that the increase in IgG4 ab occurs as a component of an overall increase in IgG ab, and it appears invalid to interpret clinical findings in terms of this subclass ab alone.

In addition to a potential blocking role, IgG4 ab has been considered to have an effector role based on a number of experimental findings. These include monoclonal anti-IgG4-dependent mediator release from peripheral blood basophils and the demonstration of an anaphylactic IgG ab, termed short-term sensitizing ab.^{8,31} By contrast, the extensive experiments of Van Toorenbergen and Aalberse³² present no support for a role for IgG4 in mediator release. Our experiments have not been designed to address this issue; however, we and others have demonstrated that IgG4 is widely distributed and can be present often at high levels in skin test negative asymptomatic individuals.⁶

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