

Serum IgG4 concentrations and allergen-specific IgG4 antibodies compared in adults and children with asthma and nonallergic subjects

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We describe the development of specific immunoassays for IgG4 protein and for allergen-specific IgG4 antibodies. We also measured the concentrations of IgG4 protein and determined the frequencies of detectable IgG4 antibodies to several common allergens in sera from adults and children with asthma and from nonallergic subjects. Serum concentrations of IgG4 protein increase with age but are not different in children with asthma and nonallergic children, nor does a raised serum concentration predict a severe clinical course in childhood asthma. IgG4 antibodies to milk and egg are common in children and adults and are more common in children with asthma than in nonallergic children less than 3 years of age. The presence of detectable IgG4 antibodies or a raised concentration of IgG4 protein in serum is not useful empirically as a diagnostic indicator of asthma but more likely results from antigen exposure occurring at mucosal surfaces. (J ALLERGY CLIN IMMUNOL 77:427-34, 1986.)

The role of antibodies of the IgG4 subclass in human allergic disease is controversial. Paraproteins of the IgG4 subclass have been reported to block PCA mediated by IgE antibodies, and IgG4 antibodies may sensitize human basophils to release histamine.¹⁻⁷ Sera from some allergic individuals contain IgG antibodies with S-TS properties.⁸⁻¹² This finding and the results above have been cited in support of the hypothesis that S-TS antibodies may be of the IgG4 isotype.¹³

The results of clinical studies also indicate that S-TS antibodies may be important in some individuals with allergic disease. The studies performed thus far have been of two types: studies that have emphasized the detection of allergen-specific S-TS antibodies in the sera of patients with allergic diseases and studies that have sought to determine a relationship between the concentrations of IgG4 protein in sera and the

Abbreviations used

S-TS: Short-term sensitizing
PCA: Passive cutaneous anaphylaxis

clinical course and response to treatment in allergic patients.¹⁴⁻²⁶ Several of these studies have focused on patients with asthma, because test for IgE antibodies are often negative and serum IgE concentrations are often normal in individuals with asthma. The results of two independent clinical studies suggest that patients with asthma and S-TS antibodies or above normal concentrations of IgG4 protein in serum respond poorly to antiasthmatic treatment with disodium cromoglycate and have a more severe clinical course.^{15, 21}

There is also evidence to suggest that IgG4 antibodies are synthesized in response to chronic exposure to antigen.²⁷⁻²⁹ Clinical studies of insect venom-sensitive individuals treated with venom immunotherapy and patients with atopic eczema have focused attention on the development of IgG4 antibodies resulting from prolonged exposure to allergens.^{30, 31} These results have led to speculation that IgG4 antibodies may act as blocking antibodies.

We have developed immunoassay methods to measure IgG4 protein and to detect allergen-specific IgG4 antibodies in serum specimens. In this study we have

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determined the range of concentrations of IgG4 protein and the frequencies and specificities of IgG4 antibodies in sera from nonallergic adults and children and from adults and children with asthma. The children with asthma were studied prospectively to determine whether raised serum concentrations of IgG4 protein or detectable IgG4 antibodies were associated with disease that was more severe clinically than in children with lower levels of IgG4 protein and no detectable IgG4 antibodies.

MATERIAL AND METHODS

Preparation and evaluation of anti-IgG4 antisera and affinity-purified anti-IgG4 antibody

Serum containing an IgG4 λ paraprotein and absorbed polyclonal monkey anti-IgG4 antisera were provided by Dr. Gerald Penn. The IgG4 λ paraprotein had been evaluated by zone electrophoresis of the isolated Fc fragment and by hemagglutination inhibition with heteroantisera to the 4a and 4b antigens.³²

Purified IgG4 λ paraprotein was obtained from serum by sequential ammonium sulfate precipitation at 30% and 60% saturation, followed by elution from diethylaminoethyl cellulose with 0.1 mol/L Tris $\cdot$ HCL with 0.2 mol/L of NaCl, pH 8.1. The eluate was further purified by gel chromatography on Sepharose 6B. An aliquot of the purified paraprotein was reduced with β -mercaptoethanol at 0.1 mol/L of final concentration for 1 hour at room temperature and then was alkylated with iodoacetamide at 0.15 mol/L, pH 8.2, on ice for 1 hour. The isolated heavy chains were recovered in the void volume from a column of Sephadex-G100 equilibrated in 0.5 mol/L propionic acid with 10^{-5} mol/L of iodoacetamide.

Antibodies were elicited in sheep by immunizing with the isolated heavy chains, 0.25 mg in complete Freund's adjuvant. The initial immunization was followed at 3 weeks by a second immunization with 5 mg of heavy chains, and subsequently at 2-week intervals by immunization with 0.5 mg of heavy chains in incomplete Freund's adjuvant. Animals were bled 1 week after each immunization. Pooled bleedings from one animal were made specific for IgG4 heavy chain antigenic determinants by sequential immunoabsorptions with preparations of Cohn fraction II pepsin digest and purified IgG1 and IgG2 paraproteins coupled covalently to Sepharose. The absorbed antiserum demonstrated a single precipitin arc on immunoelectrophoresis with purified IgG4 κ and IgG4 λ paraproteins and did not react with isolated IgG1, IgG2, or IgG3 paraproteins or with purified monoclonal κ or λ light chains.

The specificity of sheep anti-IgG4 antiserum was further evaluated by radioimmunoassay by the use of purified paraproteins and immunoglobulins of the following isotypes: IgG1, IgG2, IgG3, IgA1, and IgA2 paraproteins, polyclonal IgG from Cohn fraction II purified by diethylaminoethyl cellulose chromatography, and an IgM paraprotein purified from serum by euglobulin precipitation. The degree of cross-

reactivity was defined as the percentage of total added protein recovered as immunoreactive IgG4 as follows:

$$\% \text{ Cross-reactivity} = \frac{\text{Measured IgG4 concentration}}{\text{Total protein concentration}} \times 100$$

The specificity of sheep anti-IgG4 antiserum for polyclonal IgG4 protein was evaluated by comparing the slopes of the logit/log dose-inhibition curves of radioimmunoassays performed with an unlabeled IgG4 κ paraprotein standard and normal human serum.

Polyclonal monkey anti-IgG4 antiserum yielded a single precipitin arc when it was tested by immunoelectrophoresis versus normal human serum and sera with IgG4 paraproteins. Affinity-chromatography purified, monkey anti-IgG4 antibody was prepared by absorption and elution from a column of Sepharose-coupled IgG4 κ paraprotein. Seven milliliters of monkey anti-IgG4 antiserum was incubated with 3.5 ml of Sepharose IgG4, and the specific IgG4 antibody was eluted with 0.05 mol/L of glycine $\cdot$ HCL, pH 2.2. The resulting antibody was radiolabeled with ^{125}I by a modified chloramine-T method. The specificity of affinity-purified antibody for various IgG isotypes was evaluated by immunoradiometric assay by the use of a microcrystalline cellulose IgG4 κ paraprotein immunosorbent. The immunosorbent was prepared by coupling an immunoglobulin fraction of serum containing an IgG4 κ paraprotein to CNBr-activated microcrystalline cellulose particles. Displacement of radiolabeled, affinity-purified ^{125}I -anti-IgG4 antibody by purified IgG1, IgG2, IgG4, IgA1, IgA2, and IgM paraproteins added as inhibitors in the second stage of the assay at 1 to 100 μg per tube was measured, and the results were used to calculate cross-reactivity.

Measurement of immunoglobulin proteins and specific antibodies in serum

The concentrations of IgG4 protein in all sera were measured by a double antibody, competitive displacement radioimmunoassay. The assay was performed as follows: 100 μl of a 1:1000 dilution of sheep antiserum to IgG4, 100 μl of ^{125}I -labeled IgG4 κ paraprotein at 5 ng/ml, and 100 μl of diluted serum or purified unlabeled IgG4 κ paraprotein was added to 10 by 75 mm glass tubes. The volumes were adjusted to 850 μl with 0.1 mol/L of phosphate buffer, pH 7.5, with 10 gm/L of bovine albumin and 1 gm/L of NaN₃. After incubating overnight at 4° C, bound counts were precipitated by adding 100 μl of burro antisheep immunoglobulin antiserum and 50 μl of a 1:50 dilution of normal sheep serum. The concentrations of IgG4 protein were calculated from a logit/log transformation of the standard curve in terms of B/B₀.

The concentrations of IgE in all sera were measured by a commercial, two-site immunoradiometric assay (PRIST, Pharmacia Diagnostics Inc., Piscataway, N. J.).

Allergen-specific IgG4 antibodies in all sera were measured by immunoradiometric assays by the use of microcrystalline cellulose allergen immunosorbents.³³ Commercial allergen extracts of milk, egg, *Dermatophagoides farinae*, *Alternaria*, and short-ragweed pollen (Greer Lab-

oratories, Inc., Lenoir, N. C.) were dialyzed versus 0.15 mol/L of NaCl and coupled to CNBr-activated microcrystalline cellulose particles. The cellulose allergen immunosorbents were suspended in 0.1 mol/L of phosphate buffer, pH 7.5, with 1 gm/L of NaN₃, 10 ml/L of Tween 20, and 2 gm/L of bovine albumin (phosphate buffer) at 1 mg of cellulose per milliliter. Tests for IgG4 antibodies were performed by reacting 500 μ l of each allergen immunosorbent with 50 μ l of a 1:50 dilution of serum in polystyrene tubes after adding phosphate buffer to a final volume of 1.0 ml. After incubating overnight at room temperature, the particles were pelleted by centrifugation and washed twice. Radiolabeled, affinity-purified monkey anti-IgG4 antibody (¹²⁵I-anti-IgG4, 5 ng) was added to each tube, and the volumes were adjusted to 1.0 ml with phosphate buffer containing 40 ml/L of fetal bovine serum in place of bovine albumin. After a second overnight incubation, the tubes were centrifuged, the pellets were washed as before, and radioactivity in the pellets was measured by an automatic gamma counting system. Sera that produced binding to an allergen immunosorbent >2.5 times the nonspecific binding level in the presence of two nonallergic (negative control) sera were considered to have detectable IgG4 antibodies.

Allergen-specific IgE antibodies to the above mentioned allergens were detected in all sera by a modified radioallergosorbent test designed to screen for the presence of antibodies of several different specificities simultaneously in the same tube.³⁴ The multiallergen test was performed by the use of equal volumes of several different cellulose allergen immunosorbents incubated with 50 μ l of test serum in the first stage of the assay. IgE antibodies were detected in the second stage of the assay by adding 8 ng of ¹²⁵I-anti-IgE antibody (Pharmacia Diagnostics, Inc.) to each tube. Sera that produced binding >4 times the nonspecific binding level determined in the presence of a nonallergic serum pool were considered to have detectable IgE antibodies.

Groups of patients and control subjects

Children with asthma (n = 60). Children with asthma were recruited prospectively from patients evaluated at the Pediatric Allergy Clinic of the Mayo Clinic. All diagnoses were made clinically on the basis of signs and symptoms of asthma at the time of initial presentation. Informed consent to participate in this study was obtained from a parent or guardian in each case.

Adults with asthma (n = 62). Sera from adults with asthma were selected retrospectively from sera of Mayo Clinic patients sent to the Clinical Immunology Laboratory for measurement of IgE concentration. The diagnosis of asthma was established in each patient by the primary physician on the basis of clinical signs and symptoms.

Nonallergic children (n = 82). Sera from nonallergic children were obtained from sera sent to the Mayo Clinic blood bank for compatibility testing before surgery. The medical histories of all children were reviewed to establish that no allergic diseases had been diagnosed clinically. All children in the group had serum IgE concentrations less than the mean + 1 SD for their age.

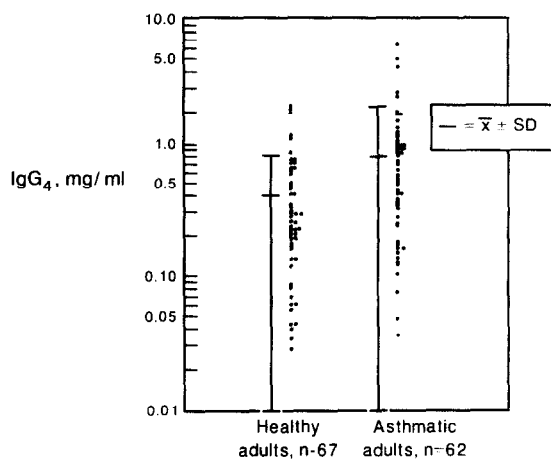


FIG. 1. Serum concentrations of IgG4 protein in nonallergic adults and adults with asthma.

Nonallergic adults (n = 67). Sera from nonallergic adults were obtained from healthy volunteers employed at the Mayo Clinic. None of the adults had clinically diagnosed allergic diseases, and all had serum IgE concentrations less than the mean + 1 SD for their age group.

Prospective study of immunoglobulin proteins and specific antibodies in children with asthma

Each child with asthma was evaluated at the time of admission to the study group and by questionnaire at monthly intervals to determine the severity of disease (symptom score) and the antiasthmatic medications required to control the clinical signs of asthma (therapeutic score). The following were determined monthly: average number of days per week with wheezing; wheezing status defined as absent, occasional, frequent, or constant; wheezing on exertion; loss of sleep caused by cough or wheezing; number of hospitalizations; number of emergency room visits for treatment of wheezing; limitation of exercise or play caused by wheezing; and the number of school days missed caused by asthma. Responses were used to calculate the symptom score for each child each month. The following parameters of therapy were also determined monthly: number of injections of β -agonists; number of days treated with oral corticosteroids; and the doses of oral theophylline, β -agonists, disodium cromoglycate, and nebulized β -agonists and corticosteroids. Responses were used to calculate the therapeutic score for each child each month. Average monthly symptom and therapeutic scores were calculated from the cumulative responses of each child during the time of the study. The relationship (prognostic significance) of serum IgG4 and IgE protein concentrations and the presence of IgG4 or IgE antibodies to the severity of disease was determined by analysis of the rank order correlations (Spearman's rho) of these serum measurements and the average symptom and therapeutic scores for the entire group of children with asthma.

TABLE I. Cross-reactivity of antisera and affinity-purified antibody to IgG4

Polyclonal sheep anti-IgG4		
Measured IgG4 concentration ($\mu\text{g/ml}$)	Total protein added ($\mu\text{g/ml}$)	Cross-reactivity (%)
IgG1	1,000	0.01
IgG2	1,000	0.02
IgG3	1,000	0.05
IgA1	3,600	< 0.01
IgA2	3,100	0.03
IgM	17,900	< 0.01

Affinity-purified monkey anti-IgG4		
Inhibition (%)	Total protein added (μg)	Cross-reactivity (%)
IgG4	1	—
IgG1	100	< 1.0
IgG2	100	< 1.0

See Material and methods for description of analytical methods.

RESULTS

Analytical characteristics of the immunoassays for IgG4 protein and IgG4 antibodies

Cross-reactivity of absorbed sheep antiserum to IgG4 with purified paraproteins of other IgG subclasses and with non-IgG isotypes was negligible (<1% for non-IgG4 subclasses) as demonstrated by the results in Table I. Excess absorbed sheep antiserum to IgG4 bound 78% of total counts of a ^{125}I -labeled IgG4 κ paraprotein standard added at 0.5 ng per tube; non-specific binding was 2.9%. The radioimmunoassay for IgG4 protein yielded parallel logit/log displacement curves in the presence of an unlabeled IgG4 κ paraprotein standard, Cohn fraction II, and human serum. A 1:1000 dilution of the antiserum detected 30.2 ng of immunoreactive IgG4 ($B/B_0 = 0.83$). The interassay coefficients of variation of measurements ($n = 27$) of IgG4 protein in control sera with 0.756 and 0.376 mg/ml were 10.1% and 8.6%, respectively.

Affinity-purified monkey anti-IgG4 antibody had negligible cross-reactivity with other IgG isotypes when the antibody was tested by immunoradiometric assay. The results are presented in Table I. Nonspecific binding of ^{125}I -anti-IgG4 to the cellulose allergen immunosorbents tested after incubating with nonimmune serum (50 μl of a 1:50 dilution) or excess IgG4 paraprotein was <2.5% of total counts for each allergen immunosorbent. Specific binding of ^{125}I -anti-IgG4 antibody to a Sepharose IgG4 immunosorbent was 42% of total counts; the binding of ^{125}I -anti-IgG4 antibody to cellulose allergen immunosorbents after

incubating with immune serum (50 μl of a 1:50 dilution) exceeded 20% of total counts. The interassay coefficients of variation of binding levels for control sera in assays ($n = 8$) for IgG4 antibodies were 11% to 40%.

IgG4 protein concentrations and IgG4 antibodies in subjects with asthma and nonallergic control subjects

The concentrations of IgG4 protein in sera from both groups of adults are illustrated in Fig. 1. The frequency distribution in adults with asthma was strongly skewed toward higher concentrations; the average concentration in adults with asthma was higher than in nonallergic adults ($p > 0.0001$, Mann-Whitney rank-sum test) (Table II). In both groups of children the average concentrations of IgG4 protein in serum were directly related to age. Among age-matched groups there were no differences in the average serum concentrations of IgG4 protein between children with asthma and nonallergic children (Table II). Adult levels of IgG4 in serum were reached in both groups between 6 and 11 years of age.

The frequencies of detectable IgG4 antibodies to milk, egg, *Alternaria*, *D. farinae*, and short-ragweed pollen in all groups are summarized in Table III. Healthy adults and adults with asthma had comparable frequencies of IgG4 antibodies to milk, egg, and *Alternaria*, but low titered IgG4 antibodies to short-ragweed pollen were more common in the sera of nonallergic adults (21% versus 2%). The titers of antibodies to milk and egg, as reflected by the percentage

TABLE II. Serum IgG4 and IgE protein concentrations

		<u>IgG4</u> (mg/ml)		<u>IgE</u> (U/ml)	
		$\bar{X}$	range	$\bar{X}$	range
Children with asthma	All (n = 60)	0.398	0.009 to 1.980	415	< 12 to 2228
	< 3 yr (n = 10)	0.064	0.009 to 0.138	414	< 12 to 2228
	3 to 6 yr (n = 7)	0.225	0.042 to 0.694	498	< 12 to 1515
	6 to 11 yr (n = 23)	0.559	0.009 to 1.980	407	13 to 1555
	11 to 16 yr (n = 20)	0.440	0.040 to 1.813	533	28 to 2228
Adults with asthma		0.968	0.036 to 6.33	628	< 12 to 12,177
Nonallergic children	All (n = 82)	0.473	0.001 to 3.529	23	< 12 to 85
	< 3 yr (n = 10)	0.035	0.001 to 0.061		
	3 to 6 yr (n = 16)	0.255	0.001 to 0.627		
	6 to 11 yr (n = 23)	0.699	0.016 to 2.921		
	11 to 16 yr (n = 33)	0.553	0.021 to 3.529		
Nonallergic adults		0.430	0.029 to 2.320	31	< 12 to 82

TABLE III. Serum IgG4 and IgE antibodies

	<u>IgG4 antibodies</u> (% Positive)				<u>IgE antibodies</u> (% Positive)
	Milk	Egg	<i>Alternaria</i>	<i>D. farinae</i>	
				Short-ragweed pollen	All allergens
Children with asthma					
All	43	53	30	5	47
< 3 yr	70	60	0	0	20
3 to 6 yr	28	70	14	0	28
6 to 11 yr	57	52	48	9	61
11 to 16 yr	25	45	30	5	50
Nonallergic children					
All	28	15	4	1	2
< 3 yr	10	20	10	0	10
3 to 6 yr	38	13	0	0	6
6 to 11 yr	30	17	0	0	0
11 to 16 yr	27	12	6	3	0
Adults with asthma	19	24	16	2	30
Nonallergic adults	19	17	17	21	2

See Material and methods for definition of positive results.

of total counts bound to these allergen immunosorbents, were higher in both groups than to the other allergen immunosorbents (data not presented). IgE antibodies to these same allergens were much more common in the sera of adults with asthma; 30% of adults with asthma had detectable IgE antibodies compared with 2% of the nonallergic adults. By comparison, children with asthma had much higher frequencies of IgG4 antibodies to milk, egg, and *Alternaria* than nonallergic children (Table III). IgG4 antibodies to milk were very common in children with asthma less than 3 years of age but were infrequent in healthy children of the same age. Similarly, among children

older than 6 years of age, IgG4 antibodies to *Alternaria* were common in children with asthma and rare in healthy children. IgE antibodies were also much more common in the sera of children with asthma; 47% of children with asthma had detectable IgE antibodies compared with 2% of nonallergic children.

Results of the prospective study of serologic tests in children with asthma are presented in Table IV. As expected, the average symptom scores and therapeutic scores were strongly correlated, indicating that more severely affected children required more and larger doses of antiasthmatic drugs than less severely affected children. The concentrations of IgE and IgG4

TABLE IV. Correlations between serum measurements and disease severity in children with asthma

	Protein		IgG4 antibodies	IgG4 antibodies
	IgE (U/ml)	IgG4 (mg/ml)	Milk	Egg
Age	0.386 ($p > 0.995$)	0.430 ($p > 0.995$)	-0.312 ($p > 0.975$)*	0.016 (NS)
IgE protein	—	0.310 ($p > 0.99$)	-0.009 (NS)	-0.052 (NS)
IgG4 protein	—	—	0.324 ($p > 0.99$)	0.323 ($p > 0.99$)
IgG4 antibodies to milk	—	—	—	0.491 ($p > 0.9995$)
IgG4 antibodies to egg	—	—	—	—
IgE antibodies	—	—	—	—
Symptom score	—	—	—	—

All correlations are in terms of Spearman's rho.

*Inverse correlation.

in serum were also strongly correlated with age. None of the serologic parameters tested except the concentration of IgE in serum were correlated with the severity of disease (average symptom score) or with the requirement for antiasthmatic drugs (average therapeutic score). There was a tendency for children with higher concentrations of serum IgE to require more and larger doses of antiasthmatic drugs, but there was no significant correlation between the serum concentration of IgE and the severity of asthmatic symptoms.

Higher serum concentrations of IgG4 were correlated with the presence of detectable IgG4 antibodies to milk and egg, but neither the serum concentration of IgG4 nor the titers of IgG4 antibodies were correlated with average symptom scores or therapeutic scores. In children with asthma the titers of IgG4 antibodies to milk were inversely related to age; older children with asthma tended to have lower titers of IgG4 antibodies to milk. Children with asthma and higher serum concentrations of IgE had higher frequencies of detectable IgE antibodies to the allergens tested.

DISCUSSION

Homocytotropic antibodies of the IgG isotype were described in animals before the discovery of IgE as a separate class of immunoglobulin.³⁵⁻³⁸ These antibodies, also called S-TS antibodies, sensitize skin for 2 to 4 hours and retain skin sensitizing activity after heating and after treatment with sulfhydryl reducing agents. S-TS antibodies in mice comprise a separate subclass of IgG that is differentiated from other subclasses by rapid anodal electrophoretic mobility. Myeloma proteins in man of the IgG1, IgG3, and IgG4 subclasses have been demonstrated to have skin sensitizing activity in the heterologous PCA test; human IgG4 but not IgG1 or IgG3 myeloma proteins inhibit the homologous skin sensitizing activity of IgE antibodies. Human IgG4 immunoglobulins also migrate with rapid anodal electrophoretic mobility, and anti-

sera and monoclonal antibodies raised to myeloma proteins of the IgG4 subclass have been reported to induce histamine release from human leukocytes.

The results of several clinical studies indicate a role for human S-TS antibodies, possibly of the IgG4 isotype, as mediators of allergic diseases. Parish⁸ reported the presence of allergen-specific, heat-stable S-TS antibodies detectable by PCA in the sera of persons with serum sickness induced by horse globulins.⁷ He later reported on the presence of S-TS antibodies, also detected by PCA, in the sera of patients with asthma induced by milk and in patients with rhinitis and asthma induced by grass pollen and *Aspergillus fumigatus*. Bryant et al.^{15, 19} reported finding S-TS antibodies specific for *D. pteronyssinus* and *A. fumigatus* antigens in the sera of 12 of 49 adults with asthma. In 10 of the 12 cases in which follow-up clinical data were available, the presence of S-TS antibodies correlated with a poor response to treatment with disodium cromoglycate. Bruynzeel and Berrens¹⁸ tested sera from a large group of patients with positive skin tests for the presence of IgE and IgG4 antibodies by the use of immunoradiometric methods. In those patients with allergies to animal danders, there was a high incidence of negative IgE scores and positive IgG4 scores. Vijay et al.²⁵ also reported on 10 patients with mold sensitivity with positive skin tests to mold extracts who had negative IgE antibody tests to molds but whose leukocytes released histamine on challenge with mold antigens or with anti-IgG4 antisera. These studies document the presence of allergen-specific IgG4 antibodies in allergic individuals and raise the possibility that IgG4 antibodies may be important mediators of allergic disease and a possible source of discordant results between skin tests and in vitro tests for IgE antibodies in some allergic patients.

Antibodies of the IgG4 subclass may be particularly important in childhood allergy. Gwynn et al.²¹ reported that children with asthma and above normal concentrations of IgG4 protein in serum had a worse

IgE antibodies	Symptom score	Therapeutic score
0.208 (NS)	-0.006 (NS)	0.156 (NS)
0.582 ($p > 0.9995$)	0.161 (NS)	0.276 ($p > 0.975$)
0.024 (NS)	0.093 (NS)	0.039 (NS)
-0.036 (NS)	0.133 (NS)	0.022 (NS)
0.022 (NS)	0.197 (NS)	0.184 (NS)
—	0.099 (NS)	0.098 (NS)
—	—	0.612 ($p > 0.9995$)

clinical course than children with asthma and above normal concentrations of IgE but normal concentrations of IgG4. Children in the former group had asthma that responded poorly to disodium cromoglycate and required corticosteroid treatment in nine of 12 cases. These results are similar to those reported by Bryant et al.^{15, 19} in adults, but each study involved relatively few patients.

Since systemic corticosteroid therapy in children may be associated with serious side effects, we considered it important to investigate whether there is an association between the concentrations of IgG4 and IgE in serum and the response to antiasthmatic treatment in children. The aforementioned studies also lead us to conclude that further clinical studies of IgG4 protein and IgG4 antibodies in subjects with asthma were indicated. In particular, we believed it important to determine the empirical usefulness of measurements of IgG4 protein and IgG4 antibodies as prognostic indicators by means of a prospective study in children with asthma.

Our results indicate clearly that serum measurements of IgG4 protein and IgG4 antibodies are not useful empirically to establish the diagnosis of asthma in adults or children, nor are they useful as a prognostic indicator of the severity of disease or the response to treatment with antiasthmatic drugs in children with asthma. In the group of children with asthma that we studied, the serum concentration of IgE was the only serologic parameter that was correlated with the clinical response to antiasthmatic therapy. This correlation, although it is statistically significant, is not likely to be medically useful for clinical decision making.

We also found that the concentration of IgG4 protein in serum is very low in infants and children less than 3 years of age and that serum levels increase progressively with age in both children with asthma and nonallergic children. This finding had not been noted by other investigators who relied on less sensitive radial immunodiffusion methods to quantitate IgG4 protein.

We and others have found that IgG4 antibodies are common in the sera of children and adults. Children with asthma have higher frequencies of IgG4 antibodies to milk, egg, and *Alternaria* than nonallergic children or adults; healthy adults have a higher frequency of low titered IgG4 antibodies to short-ragweed pollen than adults or children with asthma. Aalberse et al.²⁹ has proposed that synthesis of IgG4 antibodies results primarily from chronic exposure to antigen and that IgG4 antibodies, rather than having reagenic activity, may act as univalent blocking antibodies in patients treated with allergen immunotherapy. Our results tend to support the hypothesis that IgG4 antibodies are synthesized in response to chronic exposure to allergen. For example, we found that the frequency of IgG4 antibodies to milk in children with asthma was inversely related to age despite the fact that IgG4 protein concentrations are lower in young children.

The high frequency of IgG4 antibodies to food antigens in children and adults is noteworthy for another reason. We have demonstrated that IgG4 producing plasma cells are located primarily in mucosal-associated lymphoid tissues of the airway and gut.³⁹ If IgG4 antibodies are synthesized principally in response to chronic antigen exposure, it follows that exposure to antigen at mucosal surfaces may be a particularly potent stimulus for eliciting synthesis of IgG4 antibodies. The finding of higher frequencies of IgG4 antibodies to milk, egg, and *Alternaria* in children with asthma than in nonallergic children may be interpreted to support the theory that children with asthma have more permeable mucosal barriers to antigens than nonallergic children. Further studies are needed to clarify this point.

In summary, we have found no evidence that serum measurements of IgG4 protein or IgG4 antibodies have clinical utility for the diagnosis or prognostic assessment of asthma in children or adults. This is not to say that these antibodies may not yet be demonstrated to have activity as S-TS antibodies. There is also reason to pursue investigation of IgG4 antibodies as blocking antibodies in patients treated with allergen immunotherapy and as serum markers for antigen exposure that occurs at mucosal surfaces.

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