

THE RELATIONSHIPS AMONG
SHRIMP-SPECIFIC IgG SUBCLASS
ANTIBODIES AND IMMEDIATE
ADVERSE REACTIONS TO SHRIMP
CHALLENGE

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The relationships among shrimp-specific IgG subclass antibodies and immediate adverse reactions to shrimp challenge

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High levels of shrimp-specific IgE, in association with a positive prick test, are not always predictive of a positive, immediate response to double-blind, placebo-controlled, food challenge (DBPCFC) with shrimp. The observation that shrimp-sensitive individuals in general have increased levels of circulating shrimp-specific IgG is of interest because antigen/allergen-specific IgG subclasses have been associated with adverse reactions to foods. Therefore, this current study measured shrimp-specific IgG subclass and IgE antibodies in 31 individuals with histories of immediate, adverse reactions to shrimp immediately before DBPCFC and 20 shrimp-tolerant subjects. Individuals with a history of shrimp sensitivity had significantly raised shrimp-specific IgG2 and IgG4 compared to shrimp-tolerant individuals. Challenge-positive subjects were distinguished from subjects with negative or equivocal responses by an increased IgG2 ($p \leq 0.001$). Specific IgG4 was not raised ($p \leq 0.065$). These studies indicate that some shrimp-specific IgG subclass levels are increased in shrimp-sensitive subjects. However, none of the subclass responses were significantly predictive of a positive response to DBPCFC and therefore were not diagnostic of shrimp intolerance. (J ALLERGY CLIN IMMUNOL 1990;86:387-92.)

Earlier studies have demonstrated that IgE-mediated mechanisms are important in shrimp hypersensitivity.^{1, 2} High circulating levels of shrimp-specific IgE (RAST), in association with a positive prick skin test, were 87% predictive of a positive, immediate response to a DBPCFC with shrimp.³ However, this study population also included subjects with negative RASTs/positive DBPCFCs, or subjects with high RASTs/negative DBPCFCs.³ Thus, it is suggested that even in subjects with immediate reactions, IgE may not be the sole, pathogenic mechanism of food hypersensitivity.

Food-specific IgG, in particular IgG4, has been suggested as a mediator of food hypersensitivity,⁴ although its role is by no means clear.⁵ Recently, elevated levels of IgG4 have been associated with milk

Abbreviations used

DBPCFC:	Double-blind, placebo-controlled, food challenge with shrimp
PBS:	Phosphate-buffered saline
OD:	Optical density
mAb:	Monoclonal antibody

hypersensitivity in a proportion of milk-sensitive adults.⁶ Our previous studies have demonstrated that individuals with a history of shrimp sensitivity have higher serum levels of shrimp-specific IgG and IgA than shrimp-tolerant control subjects.^{1, 7} In addition, subjects with positive responses to DBPCFC had increased shrimp-specific IgG compared to nonresponders.⁷ To delineate further the role of IgG in shrimp allergy, the relationship of shrimp-specific antibodies of the IgG subclass to shrimp allergy and DBPCFC responses were determined.

MATERIAL AND METHODS

Study subjects

The study population was composed of 31 individuals with a history of immediate, adverse reactions after shrimp ingestion who had undergone DBPCFC with shrimp. The median age was 36 years (range, 20 to 49 years); 12 were male and 19 subjects were female. Seventy-one percent

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TABLE I. The proportion of study subjects with an IgG subclass ELISA more than background

Subclass	Shrimp-tolerant		Shrimp-sensitive		
	Atopic status		Challenge results		
	Nonatopic	Atopic	Negative	Pruritus	Positive
IgG1	6/10	7/10	6/9	11/12	9/10
IgG2	8/10	10/10	9/9	12/12	10/10
IgG3	10/10	6/10	5/9	8/12	9/10
IgG4	10/10	10/10	9/9	12/12	10/10

(22/31) were atopic defined by two or more positive skin tests to inhaled allergens and a personal history of respiratory allergy. The control group consisted of 10 atopic and 10 nonatopic, shrimp-tolerant individuals. The median age was 31 years (range, 21 to 60 years); nine were male and 11 subjects were female. Informed consent was obtained from all study participants before skin testing and donation of a blood sample. In the case of shrimp-sensitive subjects, blood samples were drawn before the DBPCFC.

Provocative challenge protocol

Briefly, challenges were completed within a single 8-hour testing period. The first phase was double-blind and placebo-controlled with either shrimp extract or placebo (PBS) administered at hourly intervals. The shrimp extract or saline was masked in 200 ml of vanilla ice cream containing 40 ml of Syralta (Emerson Laboratories, Texarkana, Texas) grape flavoring. The dosages of shrimp administered in this challenge were 1, 4, and 16 shrimp equivalents. If challenge was negative, it was followed by a second phase (an open food challenge) with 16 medium-sized boiled shrimp (approximately 64 gm of shrimp). Subjects were divided into three groups on the basis of their responses: (1) positive responders who exhibited reproducible, objective symptoms after shrimp challenge, (2) subjects who reported oropharyngeal pruritus as their only symptom after challenge with shrimp, and (3) subjects who did not respond to challenge.⁷

Shrimp-allergen extract preparation

A water-soluble extract of white shrimp (*penaeus setiferus*) was prepared as previously described.⁸ Briefly, shrimp were boiled in deionized water for 15 minutes without spices. Shrimp meat was removed from the shell, degutted, and ground in 0.1 mol/L of PBS, pH 7.2, with a Waring blender (Waring Products, New Hartford, Conn.). The slurry was stirred overnight at 4°C and centrifuged (27,500 g). The supernatant was concentrated on an Amicon YM5 (Amicon Corp., Danvers, Mass.) (molecular weight cutoff 5000) and centrifuged at 105,000 g. The final supernatant was dialyzed against PBS. The extract was aliquoted and stored at -20°C until use.

ELISA

ELISAs were carried out at room temperature with shrimp extract, mAb mouse antihuman IgG1 and IgG2

(Calbiochem-Behring, La Jolla, Calif.), mAb mouse anti-human IgG3 and IgG4 (ICN Immunobiologicals, Lisle, Ill.), and peroxidase-conjugated goat antimouse IgG (Tago, Inc., Burlingame, Calif.). All buffers and indicator systems used were ELISAmate kits (Kirkegaard & Perry Laboratories, Md.). Immulon I (Dynatech Laboratories, Alexandria, Va.) ELISA plates were coated with 10 µg per well of shrimp extract in PBS. After blocking with 1% bovine serum albumin in PBS (diluent-blocker), 100 µl of duplicate serum samples (diluted 1:10 in diluent blocker) was added. After incubating for 1 hour, the ELISA plates were washed (0.02% Tween 20 in PBS), reacted for 1 hour with diluted mAb, and again washed. Peroxidase-conjugated goat antimouse IgG (Tago, Inc.) (100 µl; 1:3000 dilution) was added for 1 hour before final washing. The amount of residual, attached enzyme was visualized with 0.1% hydrogen peroxide per 0.3 gm/L of 2,2'-azinobis (3-ethylbenzthiazoline sulfonic acid) as substrate. Color development was stopped after 20 minutes by addition of 100 µl of 1% sodium dodecyl sulfate, and the OD was determined at 405 nm.

Before use, mAbs were titrated against a serum pool from five shrimp-sensitive, DBPCFC-positive, study subjects to determine the appropriate dilution. The clones used were anti-IgG1 (HP6001 diluted 1:500), anti-IgG2 (HP6002 diluted 1:500), anti-IgG3 (SJ33 diluted 1:1000), and anti-IgG4 (SK44 diluted 1:2000).

Each study subject's serum sample was diluted 1:10 and assayed in duplicate in the ELISAs. Thus, for each serum sample, a mean OD was determined. To control for interplate variability, the assays were standardized as follows: serial dilutions (from undiluted to 1:256 [0.0039]) of the serum pool were run on each plate. From these results could be generated a "standard curve" of pooled serum dilution against OD. With this standard curve, an equivalent serum dilution was determined from the mean OD of each study subject's diluted serum sample. If a serum sample elicited a reading similar to background, it was reported as equivalent to the first of the serum pool serial dilutions that elicited no shrimp-specific activity in the ELISA. The range of background reactivity was the 99% confidence interval of the OD determined on wells containing only diluent-buffer.

Shrimp-specific IgE determination

Shrimp-specific IgE levels were determined on duplicate serum samples by RAST as previously described.⁶ Briefly,

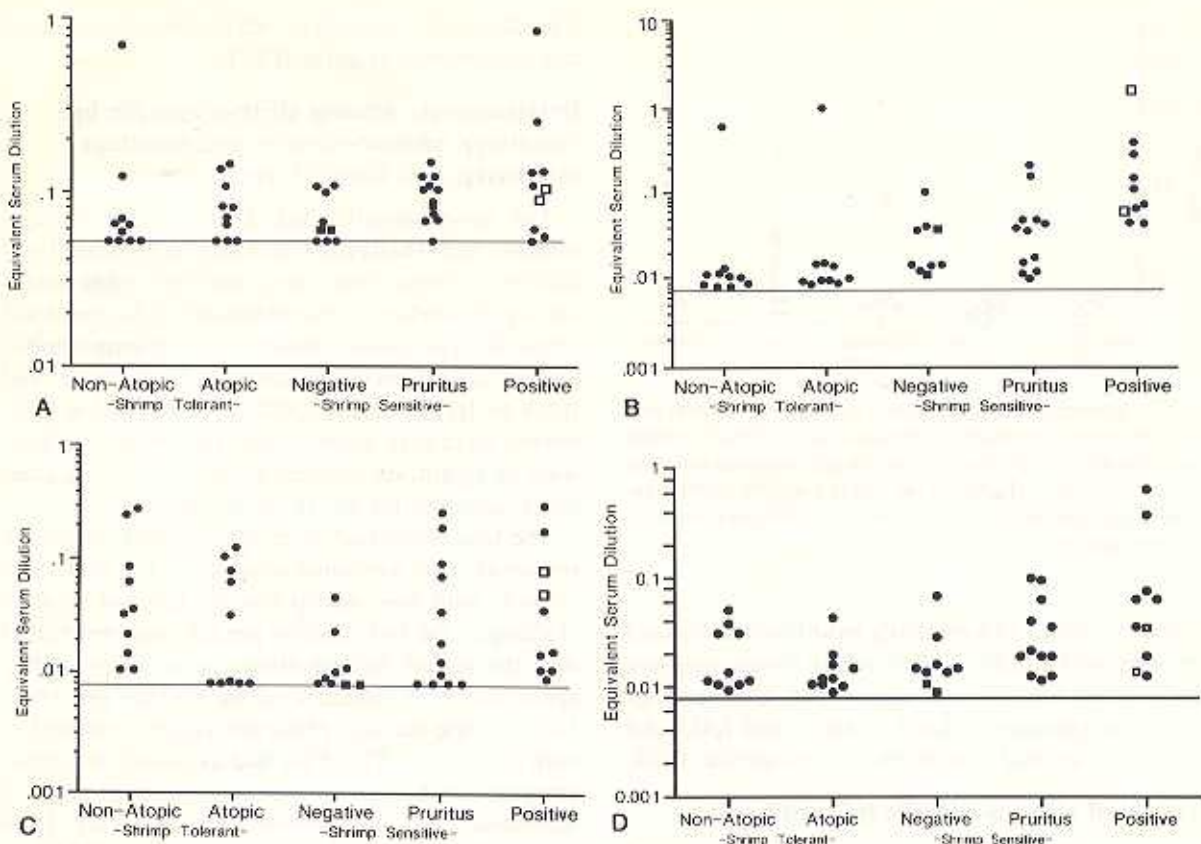


FIG. 1. Shrimp-specific IgG subclass levels grouped by clinical history of shrimp sensitivity and response to DBPCFC. **A,** IgG1 reactivity. **B,** IgG2 reactivity. **C,** IgG3 reactivity. **D,** IgG4 reactivity. Study subjects with a high shrimp-specific IgE response but negative response to challenge (■); study subjects with a positive response to challenge but no significant shrimp-specific serum IgE (□).

100 μ l of serum was added to tubes containing either shrimp-extract-coupled or human serum albumin-coupled disks, incubated overnight at room temperature on a rotator, and washed three times with 2.5 ml of physiologic saline to remove unreacted serum. One hundred microliters of 125 I-labeled anti-IgE (Kallestad Laboratories, Inc., Austin, Texas), containing approximately 25,000 cpm, was added, the incubation and washing steps were repeated, and the disks were counted on a gamma counter. Results are expressed as mean percent label bound where mean percent label bound is

$$\frac{\text{Mean cpm shrimp disks}}{\text{Total cpm}^{125}\text{I-labelled anti-IgE}} \times 100$$

RASTs were considered negative when they were $\leq 2.51\%$. This was previously determined to be the upper limit of the 99% confidence interval of the percent label bound of 13 atopic individuals nonsensitive to crustacea.⁶

Statistical analyses

The equivalent serum dilutions were compared among the study groups by the Kruskal-Wallis one-way nonpara-

metric analysis of variance (Statistix, NH Analytical Systems, St. Paul, Minn.). The ratios of shrimp-specific IgG subclass reactivities to the IgE RAST reactivity were analyzed among shrimp-sensitive study groups in a similar manner. The shrimp-specific subclass reactivities were correlated with IgE RAST levels with log-transformed data, and the significance of these correlations was determined by linear regression analysis. In all analyses, an alpha level of ≤ 0.05 was considered significant.

RESULTS

Prevalence and type of IgG subclass antibodies

With a pool of serum from study subjects with a history of shrimp sensitivity, it was possible to detect shrimp-specific antibodies in all four IgG subclasses. However, the sensitivity of the assays differed for each subclass. A 1:20 dilution was the greatest titer to yield an OD reading above background for IgG1, whereas the serum dilution was 1:64 for IgG2, IgG3, and IgG4. The proportions of individuals in each clinical

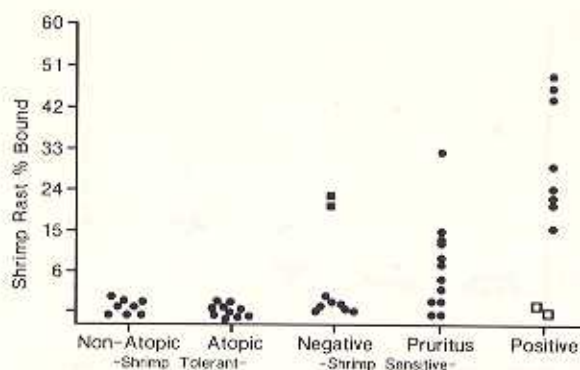


FIG. 2. Shrimp-specific IgE levels grouped by clinical history of shrimp sensitivity and response to DBPCFC. Study subjects with a high shrimp-specific IgE response but negative response to challenge (■); study subjects with a positive response to challenge but no significant shrimp-specific serum IgE (□).

category with ELISA reactivity more than background are presented in Table I. Most of both shrimp-sensitive and shrimp-tolerant individuals had OD readings above background for IgG1 to IgG2, and IgG3. All study subjects had measurable shrimp-specific IgG4.

Levels of shrimp-specific IgG antibodies

The atopic and nonatopic control populations had similar reactivities in all subclasses. Therefore, the results were expressed as a single control group for the statistical analyses.

As illustrated in Fig. 1, A to D, there was a trend toward increased shrimp-specific reactivity in the 31 individuals with a history of shrimp sensitivity in IgG1, IgG2, and IgG4, compared to 20 control subjects. The differences were significant for both IgG2 and IgG4 subclasses ($p \leq 0.0001$ and 0.0065 , respectively).

The shrimp-sensitive group was analyzed with regard to shrimp-challenge outcome. Those subjects with a positive DBPCFC demonstrated a significant elevation ($p \leq 0.0026$) in shrimp-specific IgG2 reactivity, compared both to individuals with negative responses and individuals with pruritus as their only symptom. There was no significant difference among the IgG4 levels ($p \leq 0.065$).

Shrimp-specific IgE reactivity

Of the 31 individuals with a history of shrimp sensitivity, 18 had positive RAST values ($\geq 2.51\%$), whereas 13 individuals were negative (Fig. 2). Two individuals in the challenge-positive group had negative RASTs, whereas two individuals with negative challenges had elevated RASTs (21% and 23% binding). These four individuals have been designated

("paradoxical") responders. All shrimp-tolerant, control subjects had negative RASTs.

Relationships among shrimp-specific IgE reactivity, shrimp-specific IgG subclass reactivity, and DBPCFC response

The shrimp-specific IgE levels of the shrimp-sensitive individuals correlated with levels of the IgG4 subclass. There were also positive relationships among all subclasses, except between IgG2 and IgG3 (Table II). The positive-challenge group demonstrated a trend toward decreased ratios of IgG1, IgG3, and IgG4 to IgE, whereas IgG2/IgE exhibited a trend toward increasing ratios (Table III). However, there were no significant differences among the three challenge outcomes for any of the ratios.

The four individuals with "paradoxical" challenge responses were evaluated separately. For those two subjects with low shrimp-specific IgE and positive challenges, the IgG subclass per IgE ratio was higher than the rest of their challenge group. This finding appears to be a reflection of the specific IgE level. The converse was true of the two negative responders with elevated RASTs. They had extremely low ratios compared to their group. The two challenge-negative individuals had the lowest shrimp-specific IgG4 levels. Furthermore, the individual with a RAST of 33% and pruritus as his only symptom had the lowest IgG4 value in his challenge group. One RAST-negative/challenge-positive subject had extremely high IgG2 reactivity.

DISCUSSION

Our previous studies have demonstrated that shrimp-sensitive individuals have heightened antibody responses to shrimp allergens/antigens.^{1,2} These observations have been extended by demonstrating increased shrimp-specific IgG reactivity in the IgG2 subclass and (to a lesser extent) in IgG4 subclass in shrimp-sensitive subjects. Furthermore, our results demonstrated that the DBPCFC positive subset of shrimp-sensitive individuals have significantly elevated levels of shrimp-specific IgG2 compared to shrimp-sensitive individuals with negative or equivocal (pruritus) responses to DBPCFC. When the shrimp-specific IgG and IgE reactivities were correlated, there was a significant relationship between the shrimp-specific IgE and IgG4 levels, regardless of challenge outcome.

The contribution of IgG4 to the normal immune response to food antigens, as well as its role in food hypersensitivity, has been of interest for some time. It has been proposed that elevated specific IgG4 reflects prolonged exposure to antigen occurring at the

TABLE II. Linear regression analyses among the shrimp-specific IgG subclass and IgE reactivities carried out on log-transformed data*

	IgE	IgG1	IgG2	IgG3
IgE				
IgG1	0.059			
IgG2	0.079	0.001		
IgG3	0.578	0.001	0.114	
IgG4	0.001	0.001	0.001	0.001

*Each data point is the *p* value for the regression line.**TABLE III.** The ratio of the IgG subclasses to IgE of the three food-sensitive study groups classified by response to food challenge

	Challenge results		
	Negative	Pruritus	Positive
IgG1/IgE	0.0386* ± 0.0086 (0.0026)† (0.0031)	0.0279 ± 0.0081	0.0167 ± 0.0081 (0.0452) (0.0795)
IgG2/IgE	0.0133 ± 0.0043 (0.0013) (0.0004)	0.0117 ± 0.0053	0.0562 ± 0.0494 (0.5000) (0.0360)
IgG3/IgE	0.0083 ± 0.0035 (0.0003) (0.0004)	0.0106 ± 0.0042	0.0060 ± 0.0030 (0.0293) (0.0103)
IgG4/IgE	0.0111 ± 0.0029 (0.0005) (0.0004)	0.0078 ± 0.0024	0.0058 ± 0.0018 (0.0171) (0.0104)

*Values are the means and standard error of the mean.

†Results of individuals with "paradoxical" challenge responses are presented in parentheses.

mucous membranes^{9,10} and therefore represents a normal response to dietary protein. Furthermore, an antibody response restricted to IgG4 subclass may merely reflect prolonged chronic antigenic stimulation.¹¹ In this current study of shrimp-specific antibodies, the IgG4 subclass was the only IgG subclass in which shrimp-specific reactivity was detected in all study subjects, including the control subjects. This observation supports the contention that an IgG4 response is a normal response to dietary antigens.^{9,10} However, the shrimp-sensitive subjects did have significantly elevated IgG4 responses compared to control, shrimp-tolerant individuals. Many of these shrimp-sensitive individuals claimed to have eliminated shrimp from their diet, an observation that argues against repeated exposure as the source of this reactivity. This finding is in contrast to results of egg-

allergic subjects whose elimination of egg from their diets resulted in egg-specific IgG4 reactivity that was not different from control subjects.¹²

One difficulty in evaluating food-allergic individuals is that reactions after food ingestion may be intermittent.⁸ Explanations for this phenomenon have included the possibility that non-IgE components of the immune response may modulate the occurrence of reactions. Our study was able to evaluate the relationships among shrimp-specific IgG subclasses and an objective adverse response to DBPCFC. The role of shrimp-specific IgG4 was of interest because of the literature that indicates increased levels of IgG4 antibody may protect against hypersensitivity reactions to venom^{11,13} and, in some cases, certain aeroallergens.^{13,14,15} Conversely, in food-allergic individuals, food-specific IgG4 has been associated with food in-

tolerance.^{5,6} Our results support this latter contention. For example, the challenge-positive individuals demonstrated a trend ($p \leq 0.065$) toward higher shrimp-specific IgG4 levels than did the rest of the shrimp-sensitive individuals. Furthermore, the IgG4 and IgE levels correlated and IgE levels were elevated in most positive responders.³ Finally, subjects with high shrimp RASTs and a negative DBPCFC had the lowest shrimp-specific IgG4 in the negative-challenge group. Thus, IgG4 may have a provocative role in shrimp-induced allergic reactions in contrast to its protective role in venom and respiratory allergy.

One unexpected finding of the study was the markedly elevated shrimp-specific IgG2 in shrimp-sensitive subjects. To our knowledge, the combination of elevated antigen/allergen-specific IgG2, IgG4, and IgE in a food-sensitive population has not been previously reported and may be peculiar to shrimp allergens. For example, individuals with milk-specific antibodies restricted to subclasses IgG2 and IgG4 had quite different patterns of reactivity to gliadin.¹⁰ Intriguing aspects of the IgG2 response remain to be investigated. Such investigations include the determination of the nature of the antigen interacting with IgG2^{16,17} as well as any allotype restriction of response.¹⁸

This study has demonstrated that shrimp-specific IgG subclass levels are, in general, increased in shrimp-sensitive individuals. However, no pathogenic mechanism was discerned for the RAST negative/challenge-positive patients. None of the shrimp-specific IgG subclass responses were predictive of a positive response to DBPCFC and therefore were not diagnostic of shrimp intolerance. It is worth remembering that studies of food-specific serum immunoglobulins in food allergy may be limited. It is possible that various components of the gut secretory immune system may modulate the specific response observed during food challenge. These putative factors remain to be elucidated but may shed additional light on the protective and/or pathogenic mechanisms operating in the adverse responses to foods, particularly in the "paradoxical" responders.

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REFERENCES

1. Daul CB, Morgan JE, Waring N-P, McCants ML, Hughes J, Lehrer SB. Immunologic evaluation of shrimp-allergic individuals. *J ALLERGY CLIN IMMUNOL* 1987;80:716-22.

2. Waring N-P, Daul CB, deShazo RD, McCants ML, Lehrer SB. Hypersensitivity reactions to ingested crustacea: clinical evaluation and diagnostic studies in shrimp-sensitive individuals. *J ALLERGY CLIN IMMUNOL* 1985;76:440-5.
3. Daul CB, Morgan JE, Hughes J, Lehrer SB. Provocation-challenge studies in shrimp-sensitive individuals. *J ALLERGY CLIN IMMUNOL* 1988;81:1180-6.
4. Parish W. Short-term anaphylactic IgG antibodies in human sera. *Lancet* 1970;2:591-2.
5. Stanworth D. The molecular pathology of IgG4. *Monogr Allergy* 1986;19:227-40.
6. Shakib F, Morrow Brown H, Phelps A, Redhead R. Study of IgG subclass antibodies in patients with milk intolerance. *Clin Allergy* 1986;16:451-8.
7. Daul CB, Morgan JE, Lehrer SB. The natural history of shrimp-specific immunity [in press]. *J ALLERGY CLIN IMMUNOL*.
8. Morgan JE, O'Neil CB, Daul CB, Lehrer SB. Species-specific shrimp allergens: RAST and RAST-inhibition studies. *J ALLERGY CLIN IMMUNOL* 1989;83:1112-7.
9. Homburger H, Mauer K, Sachs M, O'Connell E, Jacob G, Caron J. Serum IgG4 concentrations and allergen-specific IgG4 antibodies compared in adults and children with asthma and nonallergic subjects. *J ALLERGY CLIN IMMUNOL* 1986;77:427-34.
10. Barnes R, Harvey M, Bleas J, Finn R, Johnson P. IgG subclass of human serum antibodies reactive with dietary proteins. *Int Arch Allergy Appl Immunol* 1986;81:141-7.
11. Aalberse R, van der Gaag R, van Leeuwen J. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J Immunol* 1983;130:722-6.
12. Kemeny D, Urbanek R, Amlot P, Ciclitira P, Richards D, Lessof M. Subclass of IgG in allergic disease. I. IgG subclass antibodies in immediate and nonimmediate food allergy. *Clin Allergy* 1986;16:571-81.
13. Urbanek R, Kemeny D, Richards D. Subclass of IgG anti-bee venom antibody produced during bee venom immunotherapy and its relationship to long-term protection from bee stings and following termination of venom immunotherapy. *Clin Allergy* 1986;16:317-22.
14. Djurup R, Malling H-J. High IgG4 antibody level is associated with failure of immunotherapy with inhalant allergens. *Clin Allergy* 1987;17:459-68.
15. Nakagawa T. IgG subclass changes in response to desensitization. *Monogr Allergy* 1986;19:253-61.
16. Hammarstrom L, Persson M, Smith C. Immunoglobulin subclass distribution of human anti-carbohydrate antibodies: aberrant pattern in IgA-deficient donors. *Immunology* 1985;54:821-6.
17. Weiss J, Austin R, Schanfield M, Kagnoff M. Gluten-sensitive enteropathy. Immunoglobulin G heavy-chain (Gm) allotypes and the immune response to wheat gliadin. *J Clin Invest* 1983;72:96-101.
18. Ambrosino D, Barrus V, DeLange G, Siber G. Correlation of the Km(1) immunoglobulin allotype with anti-polysaccharide antibodies in caucasian adults. *J Clin Invest* 1986;78:361-5.