

Relationship between IgG₁ and IgG₄ antibodies to foods and the development of IgE antibodies to inhalant allergens. I. Establishment of a scoring system for the overall food responsiveness and its application to 213 unselected children

P. G. CALKHOVEN, MARJA AALBERS, V. L. KOSHTÉ, R. W. GRIFFIOEN*, J. C. VAN NIEROP*, D. VAN DER HEIDE† and R. C. AALBERSE

*Central Laboratory of The Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, *Emma Children's Hospital, Amsterdam, and*

†Department of Endocrinology, University of Leiden, Leiden, The Netherlands

Summary

To obtain reference levels for subsequent investigations, we analysed the IgG₁ and IgG₄ antibody levels to common foods in the sera of 213 unselected children (age 3 months to 14 years). The children were clustered into five age groups and tested on a broad screening panel of common foods. We used the IgG₁ and IgG₄ RAST with Sepharose-coupled antigens: cows' milk, hens' egg white, banana, legumes (a mixture of soybean and peanut), grains (a mixture of wheat and rice), potato, orange and pork. In all age groups and all antigens, a considerable variability in the antibody response was found. As for some assays more than half of the sera were negative or borderline, statistics based on interval or ordinal scaling were considered inappropriate and we resorted to nominal classification. We decided to use, for each of the assays, the 75-percentile of the age group as a cut-off level. Each antibody titre was thus converted into positive (more than the 75-percentile of that age group) or negative; the number of positive tests was used as the score. This resulted in a ΣG_1 -score and a ΣG_4 score (summed scores for IgG₁ and IgG₄ antibodies, respectively). The results of the present study indicate that children with a high response to one food tend to have elevated responses to other non-related foods, possibly explained by a defective mucosal barrier and/or a hyperactive immune system. This suggests that a high-food responder phenotype may exist.

Clinical and Experimental Allergy, Vol. 21, pp. 91-98. Submitted 28 February 1990; revised 9 July 1990; accepted 13 August 1990.

Introduction

Early in life children are exposed to a variety of environmental stimuli which may trigger a local or systemic immune response. In children with a family history of atopy, this immune response often includes the production of IgE antibodies [1,2]. Apart from a hereditary component, quantitative differences in environmental

factors may also influence the development of both IgE antibodies and allergic disease [2].

Within days of the first ingestion of cows' milk, most infants make a brisk, presumably harmless, antibody response often involving all immunoglobulin classes to multiple milk antigens [3,4]. In infants with atopic eczema and/or cows' milk allergy, the level of both IgE and IgG antibodies tends to be elevated. It has been suggested that dietary manipulation might influence the development of IgE antibodies to inhalant antigens. Moreover, Hom-burger *et al.* [5] reported that children with asthma have

Correspondence: Dr R. C. Aalberse, c/o Publication Secretariat, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, PO Box 9406, 1006 AK Amsterdam, The Netherlands.

higher frequencies of IgG₄ antibodies to milk and egg than non-allergic children.

In a follow-up study (part II of this paper), we investigated whether young children, who on the basis of family anamnesis and/or atopic eczema were considered to have an increased risk for the development of inhalant allergy, show an abnormal IgG antibody response to food.

In this paper, we present our data and data analysis related to the establishment of age-related reference values for IgG antibodies to foods.

Subjects and methods

Population

Finger-prick blood was drawn for routine intake procedures after written informed consent from the parents from 213 unselected children from the Department of Surgery of the Emma Children's Hospital, Amsterdam. The children were 3 months to 14 years old and were divided into five age groups: 0 (3–12 months; $n=21$); 1 (13–36 months; $n=44$); 2 (37–60 months; $n=47$); 3 (61–96 months; $n=53$), and 4 (97–168 months; $n=48$). For comparison, we also tested sera of 100 unselected children (age: 12–16 yr) from Helmond, The Netherlands. Pooled normal human serum (NS-6) consisted of 500 serum samples obtained from military recruits (18–21 yr).

Chemicals

All chemicals were obtained from Merck (Darmstadt, FRG), unless indicated otherwise.

Antigens

Grass pollen (*Dactylis glomerata*) was obtained from Diephuis Laboratory (Groningen, The Netherlands); dried house dust mites (*Dermatophagoides pteronyssinus*) from CSL (Melbourne, Australia); cat-dander extract and dog-dander extract from HAL (Haarlem, The Netherlands). Foods were obtained locally.

Ten per cent (w/v) extracts of grass pollen, soybean, peanut, wheat, rice, pork, cows' milk and hens' egg white were prepared with water at pH 8.0. Extracts of potato, banana and orange were prepared in potassium phosphate buffer, pH 7.5, according to Björkstén *et al.* [5] with the use of polyvinyl-polypyrrolidone (PVPP; Fluka, Buchs, Switzerland) and diethyldithiocarbamate. Extracts were defatted with freon (final concentration 33%, v/v), dialysed against water for at least 60 hr and lyophilized. The extracts were coupled to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) according to the manufacturer's instructions,

except for the inactivation, which was performed with 0.5 M glycine at pH 8.5 for 2 hr.

The amounts of extract from inhalants and foods coupled per gram solid phase were: grass pollen, 5 mg; house-dust mite, 5 ml of 3% (w/v) extract; cat dander, 2 ml of 10% (w/v) extract; dog dander, 1 ml of 10% (w/v) extract; soybean, peanut, wheat, rice, potato, banana, orange and pork, 50 mg; cows' milk, 30 mg decaseinated milk + 80 mg casein; hens' egg white, 25 mg.

Anti-immunoglobulin antibodies

Affinity-purified sheep anti-human-IgE antibodies were prepared as described previously [7]. Affinity-purified anti-human-IgG₁ antibodies (SH 161-03-51) and murine monoclonal anti-human-IgG₄ antibodies (MH 164-1-MO1) were obtained from the Department for the Production of Immune Reagents at the CLB. Anti-human-IgG₁ (50 µg) and anti-human-IgG₄ (75 µg) were labelled with 1 mCi of ¹²⁵I each as described by Hunter *et al.* [8] and subjected to gel filtration through a column of ACA-44 (LKB, Bromma, Sweden).

Assay of total IgG₄ and total IgE

Total IgG₄ was measured in a non-competitive two-site radioimmunoassay using the monoclonal antibodies MH 164-2-MO1 for capture and MH 164-1-MO1 as the detecting reagent. One milligram of antibody was coupled to 100 mg of CNBr-activated Sepharose 4B and suspended in 100 ml of PBS-A (PBS containing 3 mg bovine serum albumin/ml, Organon Teknika, Oss, The Netherlands). In each test, 250 µl of this suspension were incubated overnight with 5 µl of serum (diluted 1:200 with PBS-A), 250 µl of PBS-AT (PBS-A containing 0.2% (v/v) Tween-20 (Baker, Deventer, The Netherlands)) and 50 µl ¹²⁵I-labelled antibody in the presence of 12.5 µl of a mixture of bovine and sheep serum (9 parts bovine serum + 1 part sheep serum). After washing, the bound radioactivity was counted. Total IgG₄ was calculated from a calibration curve of the reference serum H00-03 containing 0.62 mg IgG₄/ml. Total IgE was measured as previously described [7].

IgG₁, IgG₄ and IgE RAST

In the IgG₁ RAST, 500 µl of Sepharose-coupled antigen (1 mg Sepharose/ml) were incubated overnight with 500 µl of PBS-AT and 30 µl of serum (diluted 1:40 with PBS-A). After washing, 50 µl of ¹²⁵I-labelled sheep anti-human-IgG₁ were added and incubated overnight in the presence of 500 µl of PBS-AT. After washing, the bound radioactivity was counted.

In the IgG₄ RAST, 500 μ l of Sepharose-coupled antigen (1 mg Sepharose/ml) were incubated overnight with 100 μ l of serum, previously diluted 1:40 with PBS-A. After washing, 50 μ l of ¹²⁵I-labelled murine monoclonal anti-human-IgG₄ antibodies (MH 164-1-MO2, 10 mCi/mg antibody) were added and incubated overnight in the presence of 500 μ l of PBS-AT. After washing, the bound radioactivity was counted.

Specific IgE to the inhalants, grass pollen, house dust mite, cat dander and dog dander was determined with the RAST as previously described [9].

Statistics

Statistical analysis was performed with chi-square statistics and the Kolmogorov-Smirnov test using the Statistical Package for the Social Sciences (SPSS-PC).

Results

Selection of foods for inclusion in the screening panel

To establish a suitable antigen panel for screening the IgG antibody response to common foods, sera from 18 children aged 2-4 yr were tested on a panel of 15 common foods. The sera were selected from samples submitted for routine diagnostic IgE determinations. In none of these sera were IgE antibodies, directed against cows' milk or hens' egg white, detectable. The results of these pilot IgG RASTs are given in Table 1. As shown, food-specific IgG₁ and IgG₄ antibodies were easily detected. For IgG₄ in particular, high levels of food-specific antibodies were observed (cows' milk, egg white, banana).

From the results of our pilot panel, eight foods (or combinations of closely related foods) were selected which would give the maximum number of positive results: (1) legumes (LEG; a mixture of soybean and peanut); (2) grains (GRN; a mixture of rice and wheat); (3) potato (POT); (4) banana (BAN); (5) orange (ORA); (6) pork (PRK); (7) cows' milk (MLK), and (8) hens' egg white (EGG). The selected antigens cover the normal spectrum of foods given to infants in The Netherlands.

Age-related food response

In Fig. 1, the data of the IgG₁ and IgG₄ anti-food antibody assays of the largest group tested, a cohort of 100 schoolchildren, are shown. In Fig. 2, the results are presented as 75-percentiles ($\pm$ s.e.), calculated as described by Campbell and Gardner [10]. The results were ranked according to the 75-percentile value of the IgG₁ antibody response. It is clear that the IgG₄ response to EGG and BAN is considerably stronger than expected on the basis of the IgG₁ response when compared to, for example the responses to LEG or GRN.

The same pattern was found in four of the five age groups of younger children (Fig. 3). Only in the youngest age group (group 0, consisting of children aged less than 1 yr) was this phenomenon absent. As expected, in this group only the IgG₄ response to MLK was pronounced, as was the IgG₁ response. In general, the IgG₁ antibody levels were highest in group 1 (age: 13-36 months), whereas the IgG₄ antibody levels were higher in group 2 (age: 37-60 months) than in group 1.

The relative contribution of IgG₄ to the total IgG response is given in Fig. 4. Because, in general, the IgG₂ and IgG₃ antibody responses to the foods appeared negligible (data not shown), in this figure the total IgG antibody response represents the sum of the food-specific IgG₁ and IgG₄ antibody responses. The difference in relative contribution of IgG₄ versus IgG₁ to the total IgG response for two antigens was confirmed in an antigen-binding assay using iodinated purified components of cows' milk (α -lactalbumin and β -casein) and egg white (ovomucoid; data not shown).

Classification of sera into low and high responders

Using the 75-percentiles of the age groups as the cut-off levels, for each child the individual results of the food-specific IgG₁ and IgG₄ antibody assays were dichotomized by conversion into score 1 (positive, response higher than the cut-off value) or score 0 (negative). Next, for each child the eight IgG₁ scores were summed (Σ G₁), as were the eight IgG₄ scores (Σ G₄). In Table 2, the observed scores are compared with the scores expected assuming a binomial distribution. It is clear that there is an excess of low scores and an excess of high scores. This indicates that, despite the fact that each of the foods was included in the antigen panel only when it was found to give a relatively low association with other foods in that antigen panel in the pilot study, both IgG₁ and IgG₄ responses to various foods are still significantly clustered.

When a high responder is defined as someone belonging to the upper 25-percentile for Σ G₁ or Σ G₄ sera with a score of 3 or less would be classified as low responders for Σ G₁ and Σ G₄ (actual cumulative percentages of the sera: 76.5 and 76.1, respectively).

Association of high responsiveness to foods with IgE responses to inhalant allergens

Sera were tested in the RAST for four allergens: grass pollen (*Dactylis glomerata*), house dust mite (*Dermaphagoides pteronyssinus*), cat dander and dog dander. Because of the limited amount of serum available, not all sera were tested for all allergens. The results, summarized in Table 3, indicate that there is a tendency for RAST-positive sera to have elevated Σ G₁ and Σ G₄ scores.

Table 1. Results of IgG subclass RASTs for foods (pilot panel)

Serum	IgG ₁ antibodies																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Milk	3	4	6	6	7	7	7	7	8	8	10	10	11	11	12	14	17	44 (18)
Egg	1	0	1	1	0	1	1	14	1	1	0	1	0	1	2	1	0	3 (2)
Pork	7	2	3	2	1	1	2	3	3	2	1	2	1	2	2	4	1	4 (6)
Cod	2	1	1	1	3	1	1	5	0	3	1	1	2	1	1	1	0	6 (4)
Soybean	3	2	2	1	2	2	2	1	1	3	1	1	2	2	2	1	0	3 (3)
Peanut	8	2	2	1	0	1	1	2	1	2	1	1	2	1	1	2	1	3 (2)
Pea	3	2	1	0	1	1	1	1	1	1	1	1	1	0	1	1	4	3 (3)
Rice	7	2	2	0	0	0	0	1	0	1	1	1	1	0	3	5	0	10 (4)
Wheat	7	1	3	1	1	4	1	3	3	2	1	2	2	1	9	1	0	13 (7)
Potato	3	3	4	2	2	5	3	4	2	3	3	2	1	1	4	7	1	4 (11)
Carrot	2	1	4	0	1	1	3	1	0	1	1	2	1	1	5	0	0	3 (4)
Tomato	1	0	3	0	1	1	3	1	0	0	0	1	0	0	9	1	0	2 (3)
Orange	2	1	1	1	0	0	1	1	1	2	1	2	1	0	5	2	0	2 (1)
Apple	3	2	4	1	1	2	4	1	1	3	1	4	2	1	4	1	1	5 (7)
Banana	3	3	3	1	2	9	8	4	8	7	2	3	7	3	25	3	3	5 (15)

Serum	IgG ₄ antibodies																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Milk	1	8	28	2	50	25	5	47	50	52	46	49	44	46	20	35	27	17 (16)
Egg	1	0	4	1	5	4	6	52	38	8	37	15	27	1	9	5	2	1 (12)
Pork	1	0	1	0	3	0	1	15	11	2	6	2	2	0	1	2	0	0 (4)
Cod	0	0	0	0	1	0	0	1	1	2	5	0	0	0	0	1	0	0 (1)
Soybean	1	0	1	1	6	2	1	3	8	8	2	1	2	1	4	2	0	0 (5)
Peanut	5	1	2	0	3	2	0	2	9	13	1	1	1	0	2	3	0	0 (5)
Pea	1	2	1	1	3	2	0	2	3	7	3	2	1	1	6	4	0	0 (6)
Rice	0	1	0	0	2	1	0	3	2	2	1	0	1	0	8	1	0	0 (2)
Wheat	1	1	3	1	2	27	10	46	31	1	5	1	9	0	42	2	1	0 (8)
Potato	0	2	1	0	6	1	0	11	4	2	3	2	1	0	17	5	0	0 (6)
Carrot	1	2	0	0	1	0	0	7	1	1	1	1	1	0	6	2	0	0 (2)
Tomato	1	1	0	0	0	1	0	5	1	1	2	0	1	0	4	1	0	0 (2)
Orange	1	2	3	0	1	0	0	14	3	2	29	1	2	0	14	3	0	0 (6)
Apple	0	4	2	0	4	2	0	6	2	4	10	1	2	0	31	4	1	1 (7)
Banana	38	43	45	0	22	47	36	41	51	45	20	20	55	35	57	47	16	1 (16)

Blank-corrected results expressed as percentage of bound radioactivity.

A test result is considered positive if at least 2% of radioactivity is bound. Figures in parentheses are numbers of children with positive results per food tested.

However, by chi-square analysis, the difference with the ΣG_1 and ΣG_4 scores of the RAST-negative sera is not significant.

Discussion

This paper is the first part of a study which was initiated to establish whether an enhanced immune response to foods would predispose to the development of an immune

response towards inhalant allergens. We therefore had to decide how to establish criteria by which a high response to foods could be characterized. Problems to be solved were: which foods to test and how to transform individual antibody responses into an age- and diet-independent score.

Selection of foods for the screening panel

Our aim was to establish a robust scoring system to be used in a population which is heterogeneous with respect

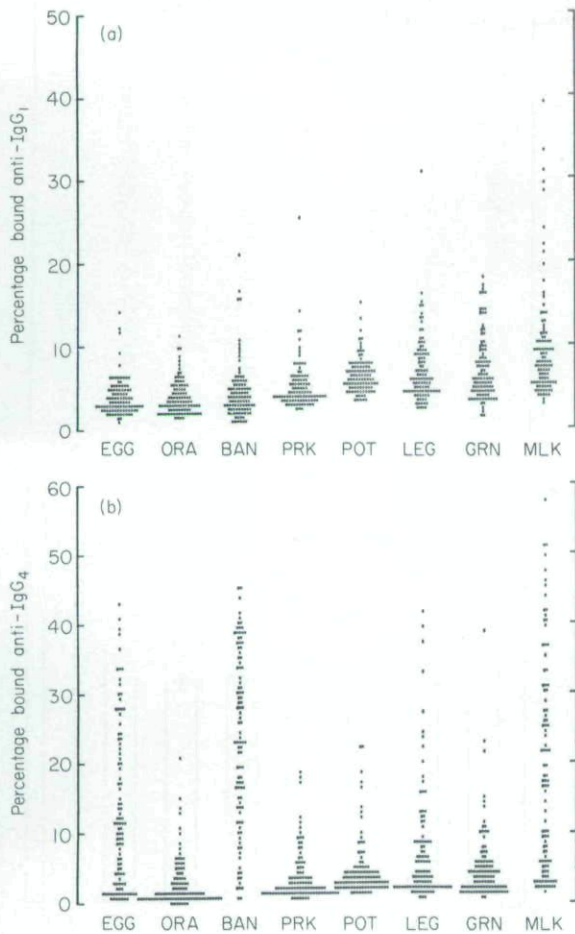


Fig. 1. IgG₁ (a) and IgG₄ (b) antibody response to foods in 100 children (12–16 yr of age).

to the types of foods consumed. The antigen panel was established on the basis of a pilot experiment in which a larger number of foods was included. For practical reasons we did not want to perform more than eight tests per serum per antibody isotype. We considered it uneconomical (in terms of maximizing the information) to test two foods separately if the antibody response to these foods was closely associated. For many of the foods tested, we occasionally observed quite specific reactivity (cf. Table 1). However, the tendency of the antibody responses to cluster was still very marked (Table 2).

With the antigen panel selected, we obtained a robust scoring system: deleting the results of any one of the foods did not result in markedly different food scores (ΣG_1 or ΣG_4). This suggests that this scoring system would be little affected by differences in diets.

The specificity of the IgG₄ binding to banana–Sephadex

The high frequency of strongly positive IgG₄ RAST (but not IgG₁ RAST) results to banana was striking. More-

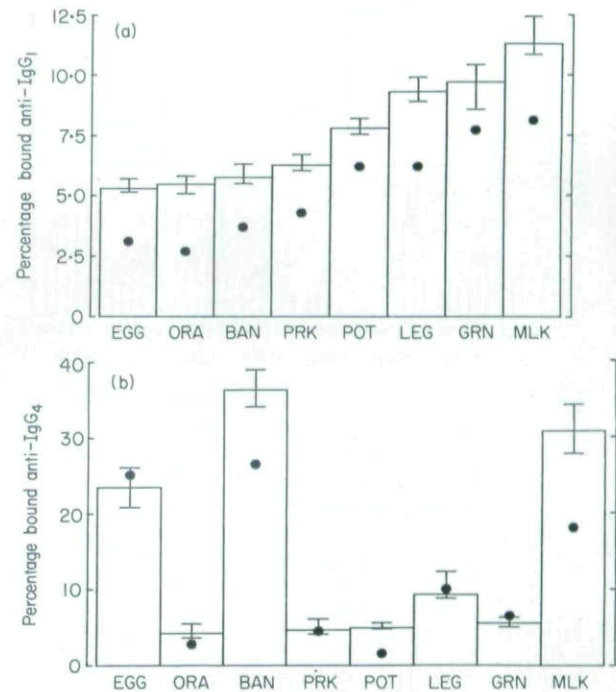


Fig. 2. IgG₁ (a) and IgG₄ (b) antibody response to foods in 100 children (12–16 yr of age). The bar height indicates the 75th percentile; the error lines indicate the standard error of the 75th percentile value. The dot in each bar indicates the value of a serum pool of normal adults; this value is closely equivalent to the mean value for normal adults.

over, a significant correlation was found between the total IgG₄ level and the results of the IgG₄ assay for banana. This clearly suggested that IgG₄ was perhaps bound via some non-immune mechanism. However, two arguments indicate that there is specificity in the binding of IgG₄ to the immobilized banana extract.

(1) Although the correlation between the total IgG₄ level and the IgG₄ assay to banana was significant (Pearson correlation coefficient 0.66, $P < 0.01$), sera were found that were high in the IgG₄ assay to banana despite a low level of total IgG₄; furthermore, some sera with high levels of IgG₄ and five purified IgG₄ myeloma protein samples tested (λ as well as κ , 4a as well as 4b) were all low in the IgG₄ RAST for banana.

(2) Absorption of serum with Sephadex-coupled banana extract removed all activity in the IgG₄ assay to banana, but had only a minor effect on the IgG₄ antibodies directed to non-related antigen.

From these and other experiments [unpublished observations], we concluded that the IgG₄ activity as measured in the banana IgG₄ RAST is largely due to genuine IgG₄ antibodies.

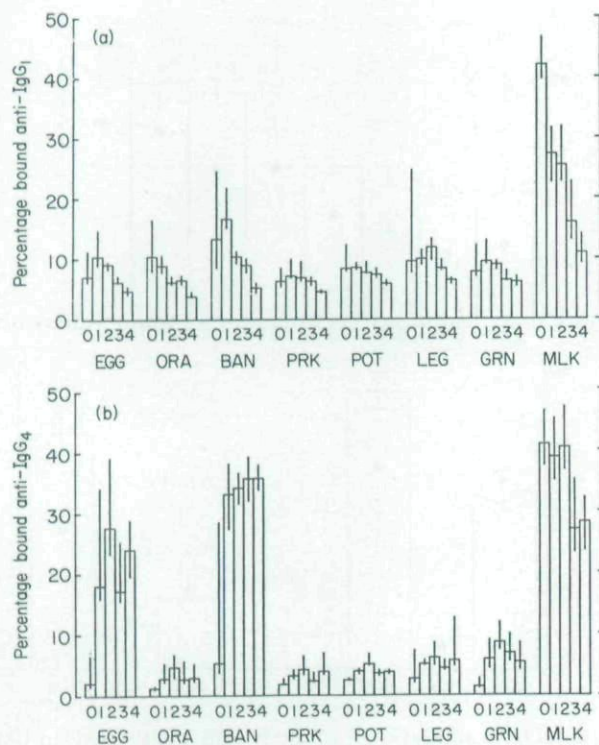


Fig. 3. IgG₁ (a) and IgG₄ (b) antibody response to foods in 213 age-clustered children. For each food, the 75-percentile of the response of the five age groups (0–4 as defined in subjects and methods) is indicated by the bar. The error-line in each bar indicates the standard error of the 75-percentile value.

The use of the 75-percentile to dichotomize the antibody response

Dichotomization of the quantitative values of the antibody assays obviously results in a loss of potentially valuable information. However, in all age groups and for all antigens, a considerable variability in the response was found. Because for some assays more than half of the sera were negative or borderline, statistics based on interval or ordinal scaling were considered inappropriate and we resorted to nominal classification. We decided to use, for each of the assays, the 75-percentile of the age group as a cut-off level that could be established with reasonable accuracy. Each antibody titre was thus converted into positive (more than the 75-percentile of that age group) or negative. Consequently, by definition, 25% of the population would be expected to have a positive response to a particular food.

Classification into low and high responders

In this way, the number of positive tests could be used as the score, with the same weight for each of the foods. This

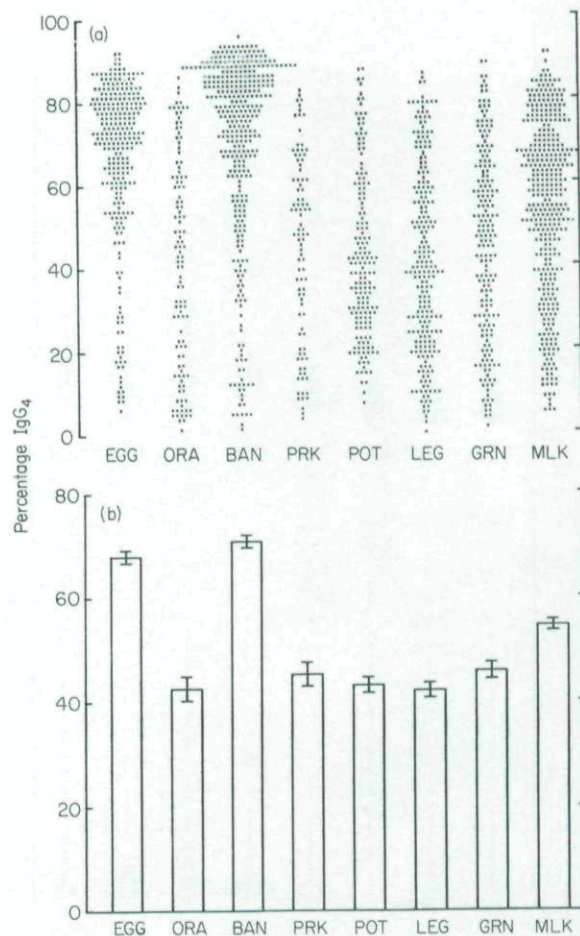


Fig. 4. Relative contribution of IgG₄ to the total IgG antibody response to foods in children aging older than 23 months. The relative contribution was calculated only for sera whose sum of the bound radiolabelled anti-IgG₁ and anti-IgG₄ was 10% or more. The data are presented as individual results (a) and as medians $\pm$ 1 s.e. of the median (b), calculated according to Campbell and Gardner [10].

resulted in a Σ G₁ score and Σ G₄ score (summed scores for IgG₁ and IgG₄ antibodies, respectively), both theoretically ranging from 0 to 8. If one defines a high responder as someone having a score above the 75-percentile (i.e. 25% of the population would by definition be high responders), a high responder child will have at least four positive food-specific IgG₁ or IgG₄ tests (i.e. Σ G₁ or Σ G₄ score at least 4; cf. Table 2).

Could the food antigens be combined to give a single test for a food mixture?

The major difference between a combined test and the scoring system we put forward would be that strongly

Table 2.

Score	Frequency		
	Expected ΣG_1 or ΣG_4	Observed ΣG_1	Observed ΣG_4
0	28.43	79	80
1	66.34	31	41
2	66.34	31	24
3	36.86	22	18
4	12.29	20	15
5	2.46	12	12
6	0.27	11	12
7	0.01	5	6
8	0.00	2	5

Using the Kolmogorov-Smirnov test, the observed frequencies of ΣG_1 and ΣG_4 scores are significantly different from those expected for a binomial distribution ($P < 0.01$). This indicates that negative tests tend to cluster, as do positive tests.

immunogenic foods like cows' milk, or (for IgG₄) egg white or banana, would in most cases highly (if not completely) determine the test result. In contrast, in the proposed scoring system, a relatively strong reaction to a weakly immunogenic food (in absolute terms therefore presumably still a weak response) has the same statistical weight as a strong response to, for example cows' milk. This makes the score less dependent on a single food and thus also less dependent on dietary differences.

Unselected versus non-allergic reference values

The reference values were established with a group of children which are assumed to be representative of the Dutch population. We aimed particularly at avoiding either positive or negative selection with regard to atopic conditions. In this respect, we considered this a group of unselected children. This is clearly different from establishing *normal* values (i.e. values for non-allergic children): our reference group undoubtedly included atopic children. We chose not to attempt to obtain values for non-atopic controls, because we were unable to exclude that a child would in the future develop atopic symptoms. Even if we would have excluded all children with atopic symptoms and/or a positive family history at the time of blood sampling, we would still be uncertain how many children in the group would develop sensitization in the future. We assumed this might be of importance, because our overall aim was to use these reference values for *prediction* of the development of sensitization towards inhalant allergens. However, as is indicated in Table 3, the influence of pre-existing inhalant allergy is only small, and similar results would be expected for a reference group consisting of non-allergic children.

Differences in isotype distribution: why do some foods give relatively more IgG₄ antibodies?

It is intriguing that in the IgG antibody response to egg white and banana, the IgG₄/IgG₁ ratio is much higher than is found in the response to, for example cows' milk or grains. Because IgG₄ responses seem to require more extensive antigenic stimulation [11-16], one hypothetical

Table 3. Association between total IgE, total IgG₄ or IgE antibodies to grass pollen, house-dust mite, cat dander or dog dander and the ΣG_1 and ΣG_4 score

Test	n	Test + (%) [*]	ΣG_1 positive			ΣG_4 positive		
			Test - (%) [†]	Test + (%) [‡]	P [§]	Test - (%)	Test + (%)	P
Total IgE	313	24.9	23.0	23.1	0.89	21.3	29.5	0.18
Total IgG ₄	313	25.2	23.0	22.8	0.92	15.7	45.6	0.00
RAST grass	302	9.3	22.3	32.1	0.34	22.6	28.6	0.63
RAST mite	311	14.8	22.6	28.0	0.96	22.3	26.1	0.70
RAST cat	277	3.6	23.2	30.0	0.91	22.8	20.0	0.86
RAST dog	271	2.2	22.9	50.0	0.29	22.6	33.3	0.90

^{*} Indicates the percentage of sera with a positive test: i.e. with a test result higher than the age-adjusted 75-percentile for total IgE or total IgG₄, or a more than 2% specific binding of anti-IgE in the RAST.

[†] Indicates the percentage of sera with a negative test and a positive ΣG_1 score.

[‡] Indicates the percentage of sera with a positive test and a positive ΣG_1 score.

[§] Indicates the statistical significance as calculated from the 2 × 2 table by chi-square analysis.

explanation might be that the antigens in egg white and banana would be more resistant to gastro-enteric digestion and/or would be more easily resorbed (perhaps because of higher glycosylation) and thus present a stronger immunogenic stimulus to the immune system.

Acknowledgments

The authors express their gratitude to Prof. T. E. W. Feltkamp and Dr C. Aaij, Dr S. O. Stapel and Dr T. A. Out for reviewing the manuscript, to Dr A. Vlug for providing the purified IgG₄ myeloma proteins, and to the NIZO (Ede, The Netherlands) for providing the α -lactalbumin and β -casein. We also gratefully acknowledge the co-operation of the nurses and the staff of the Emma Children's Hospital, Amsterdam, for collecting the serum samples of young children.

This study was financially supported by The Netherlands Asthma Foundation (grant no. 83.26).

References

- 1 Dannaeus A, Johansson SGO, Foucard T, Ohman S. Clinical and immunological aspects of food hypersensitivity in childhood. I. Estimation of IgG, IgA and IgE antibodies to food antigens in children with food allergy atopic dermatitis. *Acta Paediatr Scand* 1977; 66:31-7.
- 2 Wüthrich B, Baumann E, Fries RA, Schnyder UW. Total and specific IgE (RAST) in atopic twins. *Clin Allergy* 1981; 11:147-54.
- 3 Bahna SL. Control of milk allergy: a challenge for physicians, mothers and industry. *Ann Allergy* 1978; 41:1-12.
- 4 Dannaeus A, Johansson SGO. A follow-up study of infants with adverse reactions to cows' milk. I. Serum IgE, skin test reactions and RAST in relation to clinical course. *Acta Paediatr Scand* 1979; 68:377-82.
- 5 Homburger HA, Mauer K, Sachs MI, O'Connel EJ, Jacob GL, Caron JC. Serum IgG₄ concentrations and allergen-specific IgG₄ antibodies compared in adults and children with asthma and non-allergic subjects. *J Allergy Clin Immunol* 1986; 77:427-34.
- 6 Björkstén F, Halmepuro L, Hannuksela M, Lahti A. Extraction and properties of apple allergens. *Allergy* 1980; 35:671-7.
- 7 Aalberse RC, Brummelhuis HGJ, Reerink-Brongers EE. The purification of human polyclonal IgE by immunosorption. *Immunochemistry* 1973; 10:295-303.
- 8 Hunter WM, Greenwood FC. Preparation of iodine-131-labelled growth hormone of high specific activity. *Nature* 1962; 194:495-6.
- 9 Aalberse RC, Koshte VL, Clemens JGJ. Immunoglobulin E antibodies that cross-react with vegetable foods, pollen and *Hymenoptera* venom. *J Allergy Clin Immunol* 1981; 68:356-64.
- 10 Campbell MJ, Gardner MJ. Calculating confidence intervals for some non-parametric analyses. *Br Med J* 1988; 296:1454-6.
- 11 Aalberse RC, 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 Djurup R. The subclass nature and clinical significance of the IgG antibody response in patients undergoing allergen-specific immunotherapy. *Allergy* 1985; 40:469-86.
- 13 Iskander R, Das PK, Aalberse RC. IgG₄ antibodies in Egyptian patients with schistosomiasis. *Int Arch Allergy Appl Immunol* 1981; 66:200-7.
- 14 Merrett J, Barnetson RStC, Burr ML, Merrett TG. Total and specific IgG₄ antibody levels in atopic eczema. *Clin Exp Immunol* 1984; 56:645-52.
- 15 Ottesen EA, Skvaril F, Tripathy SP, Poindexter RW, Hussain R. Prominence of IgG₄ in the IgG antibody response to human filariasis. *J Immunol* 1985; 134:2707-12.
- 16 van der Zee JS, van Swieten P, Aalberse RC. Serologic aspects of IgG₄ antibodies. II. IgG₄ antibodies form small, nonprecipitating immune complexes due to functional monovalency. *J Immunol* 1986; 137:3566-71.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.