

Relationship between IgG₁ and IgG₄ antibodies to foods and the development of IgE antibodies to inhalant allergens. II. Increased levels of IgG antibodies to foods in children who subsequently develop IgE antibodies to inhalant allergens

P. G. CALKHOVEN, MARJA AALBERS, V. L. KOSHTE, P. P. M. SCHILTE*, J. L. YNTEMA*, R. W. GRIFFIOEN†, J. C. VAN NIEROP†, A. P. ORANJE‡ and R. C. AALBERSE

*Central Laboratory of The Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, *Medical Centre Alkmaar, Alkmaar; †Emma Children's Hospital, Amsterdam, and ‡University Hospital Rotterdam/Sophia Children's Hospital, Rotterdam, The Netherlands*

Summary

In the present investigation we have tested the hypothesis that children with a high IgG antibody response to foods have an increased risk of developing IgE antibodies to inhalant allergens. Sera from 106 children with an increased risk of developing IgE-mediated allergy were analysed. During the follow-up, in 54 of these children IgE antibodies to inhalant allergens appeared. A positive/negative IgG₁ and IgG₄ anti-food score was determined as described previously: sera from age-clustered unselected children were tested for the levels of IgG₁ and IgG₄ antibodies to common foods. For each IgG RAST and each age group, the 75-percentile was chosen as cut-off value. Each antibody level was thus converted into a positive (higher than the 75-percentile of the age group) or negative value. The number of positive tests was used as the score. High-risk children with a high IgG₁ anti-food score more often developed inhalant-specific IgE antibodies than high-risk children with low IgG₁ titres: 50% of the children with a high IgG₁ anti-food score developed IgE antibodies to grass pollen, whereas only 16% of the children with a low IgG₁ anti-food score acquired IgE anti-grass pollen. Fifty per cent of the children with a high and 14% of the children with a low IgG₁ anti-food score developed IgE antibodies to cat dander. For the prediction of the development of IgE anti-mite (house dust mite), the IgG₄ anti-food scores appeared less useful than the IgG₁ anti-food scores; 46% of the IgG₄ high responders versus 22% of the IgG₄ low responders acquired IgE anti-mite, whereas for IgG₁ these percentages were 73 and 19, respectively.

Clinical and Experimental Allergy, Vol. 21, pp. 99–107. Submitted 28 February 1990; revised 9 July 1990; accepted 13 August 1990.

Introduction

Many retrospective as well as prospective studies have been performed to unravel the relationship between nutrition and allergic disorders. Fetal sensitization

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

towards food antigens, leading to an IgE immune response, can occur when pregnant women eat some foods in excess [1–5]. Breast-feeding was proposed as a general preventive measure against allergic onset. Breast milk offers several undisputed advantages over cows' milk, e.g. it protects against gastrointestinal and respiratory tract infections [6]. However, its preventive role in allergic disorders is not clarified [1,7–9]. The delayed introduction

of solid food has been associated with a significant prevention of atopic diseases [10,11].

Because some inhalants, e.g. pollen of grasses, trees and weeds, show immunological crossreactivity with foods [12,13], we investigated the hypothesis that immunological priming towards foods predisposes to the development of inhalant allergy. We used the IgG₁ and IgG₄ antibody responses to foods as an indicator of immunological priming.

In a previous paper [14], we established an age- and (to some extent) diet-independent scoring system for IgG₁ and IgG₄ responsiveness to foods. The IgG₁ as well as IgG₄ antibody response to eight foods was determined. Using the 75-percentile for the corresponding age as the cut-off value, each IgG₁ and IgG₄ response was dichotomized by conversion into score 1 (positive, response higher than the cut-off value) or score 0 (negative). Children with four or more positive responses for either IgG₁ or IgG₄ were considered IgG₁ or IgG₄ high responders. Applying these criteria to an unselected reference group, 25% of the children were high responders [14].

In the present follow-up study, we investigated whether young children, who on the basis of family history and/or atopic eczema were considered to have an increased risk of IgE-mediated allergy, show an abnormal IgG antibody response to foods prior to the development of IgE antibodies to inhalant allergens.

Subjects and methods

Population

Young children with an increased risk of developing IgE antibodies to inhalant allergens were studied in a follow-up project. The parents were informed about the objectives of this study and gave their consent.

The children (aged between 3 months and 8 yr) were selected by the paediatricians in our group. The parents were asked to let their child participate if their child had (1) a positive family history of atopy, and/or (2) atopic eczema, and/or (3) IgE antibodies to foods; furthermore, (4) the RAST had to be negative for at least three of the five inhalant allergens tested (grass pollen, house dust mite and dander from cat, dog and rodent).

Two or more serum samples were collected from 106 high-risk children. The median ages were 27 months at enrolment and 52.5 months at the last follow-up. The median follow-up time was 18 months. The median age at enrolment of the children who had a high IgG₁ anti-food score was 22 months, for the other children 30 months. The median age at enrolment of the children who had a high IgG₄ anti-food score was 25 months, for the other children 29.5 months. During the period of follow-up,

Table 1. Frequency of IgE antibodies in the present high-risk group

	Active	
	A1	A2
Any RAST	13	41
Grass	22	6
Mite	22	23
Cat	18	15
Dog	10	20
Rodent	11	4

For each row, the total number of children was 106.

In the active group, IgE antibodies to the indicated inhalants were absent in the first serum sample, but were detected in later serum samples. In this group, two subpopulations can be distinguished.

A1: children whose first serum sample did not contain detectable IgE antibodies to any inhalant; all IgE antibodies to inhalant allergens arose during the follow-up; and

A2: children who developed IgE antibodies to the indicated inhalant; however, in contrast to subgroup A1, IgE antibodies to other inhalants were already present in the first serum sample.

54 children (20 female) developed IgE antibodies to one or several of the above-mentioned inhalant allergens. This group will be referred to as group A ('active'). The group of IgE sero-convertants had IgE antibodies to one or more inhalants which were not detected in the first serum sample. In this group, two subpopulations can be distinguished:

A1: children whose first serum sample did not contain detectable IgE antibodies to any inhalant; all IgE antibodies to inhalant allergens arose during the follow-up ($n=13$); and

A2: children whose first serum sample already contained IgE antibodies to inhalant allergens; in addition, IgE antibodies to other inhalants arose during the follow-up ($n=41$).

The specificity of IgE antibodies to inhalant allergens in these children is indicated in Table 1.

A second group consisted of high-risk children who had no IgE antibodies to inhalant allergens at the start of the investigation and did not develop IgE antibodies to inhalant allergens during the observation period; this group will be referred to as group I ('inactive'; $n=52$, 17 female).

Classification of atopic eczema

From all children the clinical history with respect to atopic eczema was obtained. The criteria for atopic

eczema were based on Hanifin and Rajika [15]. The following classification of atopic eczema was made: score 0 = no eczema ($n = 42$; 19 female); score 1 = mild eczema, but doubtful whether it was atopic ($n = 4$; 2 female); score 2 = definite atopic eczema ($n = 55$; 15 female). In five cases (2 female), no suitable data with respect to the eczema history were available.

In the active group A, the eczema was more frequent (for scores 0, 1 and 2: $n = 10$, 2 and 39, respectively) than in the inactive group I (for scores 0, 1 and 2: $n = 32$, 2 and 16, respectively).

Chemicals

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

Extracts

All (immobilized) extracts prepared and described previously [14] were also used in the present study.

The inhalant allergens tested were: grass (*Dactylis glomerata*) pollen (GRASS), *Dermatophagoides pteronyssinus* (MITE), cat dander (CAT), dog dander (DOG) and a mixture of rabbit and guinea-pig dander (RODENT).

Rabbit dander and guinea-pig dander extracts (10% w/v) were obtained from HAL (Haarlem, The Netherlands). For rodent dander, a mixture of 10 ml of guinea-pig dander extract and 4 ml of rabbit dander extract were coupled to 1 g of CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) as previously described [14].

The foods tested were: hens' egg white (EGG), orange (ORA), banana (BAN), pork (PRK), potato (POT), a mixture of legumes (soy bean and peanut, LEG), a mixture of grains (wheat and rice, GRN) and cows' milk (MLK).

Assays

Total IgE, specific IgE to inhalant allergens, total IgG₄ and the IgG₁ and IgG₄ assays to a panel of eight foods or food mixtures were performed as previously described [14].

Classification of results

The results of the radioimmunoassays were dichotomized 1 (positive) or 0 (negative) by comparing the results with those of a population of age-clustered unselected children using the 75-percentile as cut-off [14]. The scores for the eight foods tested were added to obtain a summed IgG₁ score (ΣG_1) and a summed IgG₄ score (ΣG_4), both

theoretically ranging from 0 to 8. For approximately 25% of the children, both the ΣG_1 and ΣG_4 score was 4 or more. A score of 4 or higher was used to classify a child as a high responder.

Statistics

Statistical analysis was performed using chi-square statistics including relative risk and 95% confidence intervals [16] as calculated by the Statistical Package for the Social Sciences (SPSS-PC); the correction for continuity according to Yates was applied. The effect of eczema on the observed association between IgG antibodies to foods and the development of IgE antibodies was analysed further by calculating partial correlation coefficients from the phi values of the 2×2 tables [17].

Results

In the present group of high-risk children as a whole, compared to the unselected group studied previously [14], no, or only modest increases in the percentage of children with an elevated response to foods were found (Table 2). However, as indicated in Table 3 (columns 5 and 8) and in Fig. 1, a clear-cut distinction with respect to the chance of later developing IgE anti-inhalants was found between children with low IgG anti-food scores and children with high IgG anti-food scores.

In general, IgG₄ antibodies to foods were not better predictors than IgG₁ antibodies to foods (Fig. 2); in fact, for IgE antibodies to house dust mite, the IgG₁ anti-food response was a better predictor than the corresponding IgG₄ response ($P < 0.001$).

In Fig. 3, we analysed how the IgG response to individual foods was related to the risk of developing IgE antibodies to inhalant allergens. The results suggest that for grass pollen, house dust mite and cat dander especially, the IgG₁ anti-LEG as well as IgG₁ to GRN and EGG are suitable as predictors. In general, the relative risk scores for IgG₄ as predictor were lower than those for IgG₁.

In Fig. 4, the frequencies of high IgG₁ and IgG₄ anti-food responses were analysed in relation to the presence or absence of (a history of) atopic eczema. In this analysis, only children with eczema scores 0 (no eczema) or 2 (definite atopic eczema) were included. As expected, for most of the foods tested, high IgG₁ and IgG₄ antibody responses were found more frequently in children with eczema score 2 than those with score 0. In addition, children with eczema score 2 had higher levels of total IgE ($P < 0.001$). However, elevated total IgG₄ levels were not observed more frequently in children with (a history of) atopic eczema than in children without eczema.

Table 2. Percentage of children in the present high-risk group with elevated levels (adjusted for age) of total IgE, total IgG₄, high IgG₁ or IgG₄ anti-food scores, or a high score towards individual food antigens compared to our reference group

	Percentage of children with elevated antibody levels (age-adjusted)	
	Present high-risk group (n = 106)	Unselected reference group [14] (n = 213)
Total IgE	60.4	24.9
Total IgG ₄	21.7	25.4
ΣG ₁	18.9	23.5
ΣG ₄	27.4	23.9
IgG ₁		
EGG	30.2	24.9
ORA	20.8	24.4
BAN	21.7	24.4
PRK	6.6	24.4
POT	14.2	23.9
LEG	40.6	24.4
GRN	17.9	23.9
MLK	24.5	24.9
IgG ₄		
EGG	38.7	24.4
ORA	21.7	24.9
BAN	16.0	24.4
PRK	22.6	23.9
POT	29.2	24.9
LEG	37.7	23.9
GRN	35.8	25.4
MLK	19.8	24.4

In an unselected population, an incidence of approximately 25% is expected for each of these parameters [14].

The IgG anti-food levels tended to be higher in the eczema group, but the effect was significant only for IgG₁ ($P < 0.05$).

For all five inhalant allergens investigated, the frequency of (a history of) atopic eczema was higher in the active group than in the inactive group (Fig. 5). Therefore, we analysed whether the difference between the group A and group I with respect to the IgG anti-food response could be due to the association with atopic eczema.

Table 4 contains data similar to those in Table 3, but includes in the analyses only children with definite atopic eczema. A difference between the active and the inactive group with respect to the IgG anti-food responses was still found. However, the observed difference was often not

statistically significant as determined by chi-square analysis, most likely because of the small number of subjects.

Furthermore, the effect of eczema on the observed association between IgG antibodies to foods and the development of IgE antibodies was analysed by calculating partial correlation coefficients from the phi values of the 2×2 tables [17]. In this way, a statistical correction was made for the contribution of atopic eczema to the observed association between the inhalant-specific IgE antibody response and the IgG antibody response to foods (Fig. 6). The results indicate that the association between the IgG anti-food responses and the development of IgE antibodies to inhalants was to a small extent only due to the association between IgG to foods and atopic eczema on the one hand and between atopic eczema and the development of IgE to inhalant allergens on the other.

Discussion

The primary aim of the present study was to investigate a possible relationship between a general food hyperresponsiveness and the subsequent development of IgE antibodies to inhalant allergens. We found a clear effect using this general food hyperresponsiveness as a parameter.

In this discussion, we will focus on three questions: (1) is the IgG₄ antibody response to foods a better predictor for the development of IgE to inhalant allergens than the corresponding IgG₁ antibody response?; (2) is the observed association between IgG anti-foods and the development of IgE to inhalant allergens due to an association of these two parameters with atopic eczema?; and (3) is the observed association between IgG to foods and the development of IgE to inhalant allergens a non-specific effect, e.g. due to an increased permeability of the mucosal membranes; or, alternatively, is immunological specificity involved?

The role of IgG₄

The role of IgG₄ antibodies in human allergic disease is controversial and has given rise to much speculation. In several studies it has been shown that the total serum IgG₄ level was elevated in patients with atopic eczema [18–20]. In contrast, Homburger *et al.* [2] reported that total IgG₄ levels were not different in children with asthma and non-allergic children. They stated that the presence of detectable IgG₄ antibodies or a raised concentration of total IgG₄ is not useful as a diagnostic indicator of asthma but more likely results from antigen exposure occurring at mucosal surfaces. Björkstén *et al.* [22] did not find any

Table 3. Association between IgG antibody response to foods and the development of IgE antibodies to inhalant allergens

RAST	n*	n (A1)†	IgG ₁			IgG ₄		
			$\Sigma G_1 - (\%) \ddagger$	$\Sigma G_1 + (\%) \S$	P**	$\Sigma G_4 - (\%)$	$\Sigma G_4 + (\%)$	P
Any RAST	65	13	15.3	66.7	0.014	16.1	44.4	0.127
Grass	100	22	15.9	50.0	0.004	13.3	48.0	0.001
Mite	83	22	19.4	72.7	0.001	22.1	46.7	0.103
Cat	91	18	14.3	50.0	0.007	14.3	38.1	0.037
Dog	86	10	9.6	23.1	0.353	6.0	31.6	0.008
Rodent	102	11	9.5	16.7	0.640	7.9	19.2	0.214

* Indicates the number of children RAST negative for the indicated inhalant allergen at the start of the follow-up study.

† Indicates the number of children who developed IgE antibodies to the indicated inhalant allergen during the study, being RAST negative for the other tabulated allergens at the start of the follow-up study (subgroup A1).

‡ Indicates the percentage of children with low IgG₁ anti-foods who developed a positive RAST for the indicated inhalant allergen.

§ Indicates the percentage of children with high IgG₁ anti-foods who developed a positive RAST for the indicated inhalant allergen.

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

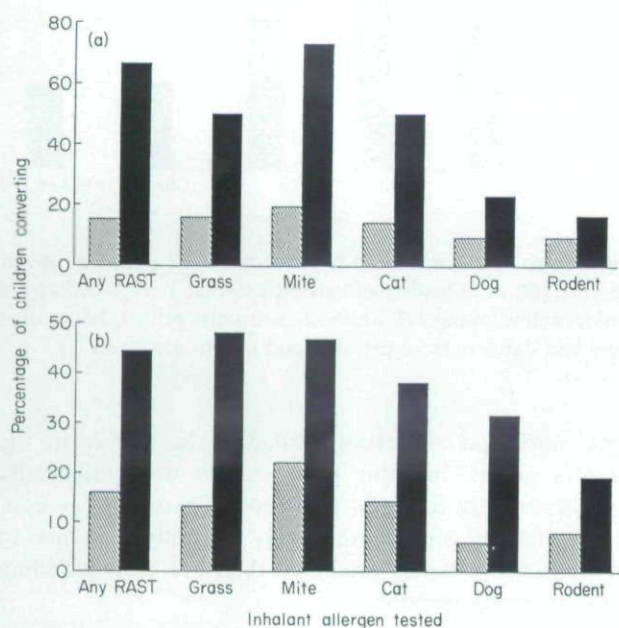


Fig. 1. Summed IgG₁ (a) and summed IgG₄ (b) antibody responses to foods (see Subjects and methods) predicting the development of IgE antibodies to inhalant allergens. For several allergens, the percentage of children showing IgE seroconversion is given. Based on the scoring system described in the first part of our study [14], serum samples from children were classified as $\Sigma G_1 -$ or $\Sigma G_4 -$ positive (■) (at least four out of eight food-specific IgG₁ or IgG₄ RASTs positive in comparison with age-clustered (unselected children) or negative (▨)).

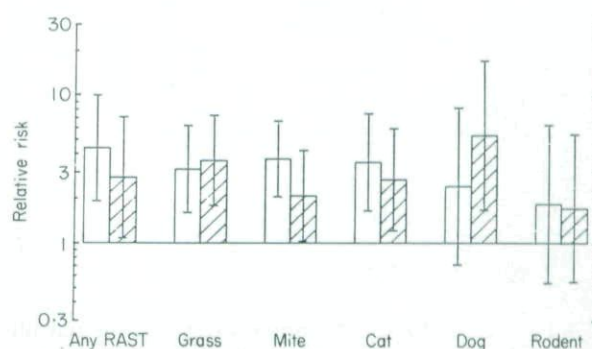


Fig. 2. Summed IgG₁ (□) and summed IgG₄ (▨) antibody responses to foods (see Subjects and methods) predicting the development of IgE antibodies to inhalant allergens. Results are expressed as relative risk (95% confidence interval).

evidence for cows' milk-specific IgG₄ antibodies playing a role in children with cows' milk allergy. In addition, Shakib *et al.* [23] concluded that the detection of milk- and egg-specific IgG₄ in patients with atopic eczema had no clinical value.

Our results do not indicate that IgG₄ is more closely associated with atopy than IgG₁. In general, for the prediction of development of IgE antibodies to any of the inhalant allergens tested, the IgG₁ antibody response to foods appeared more informative than the corresponding IgG₄ antibody response. For the prediction of IgE sero-

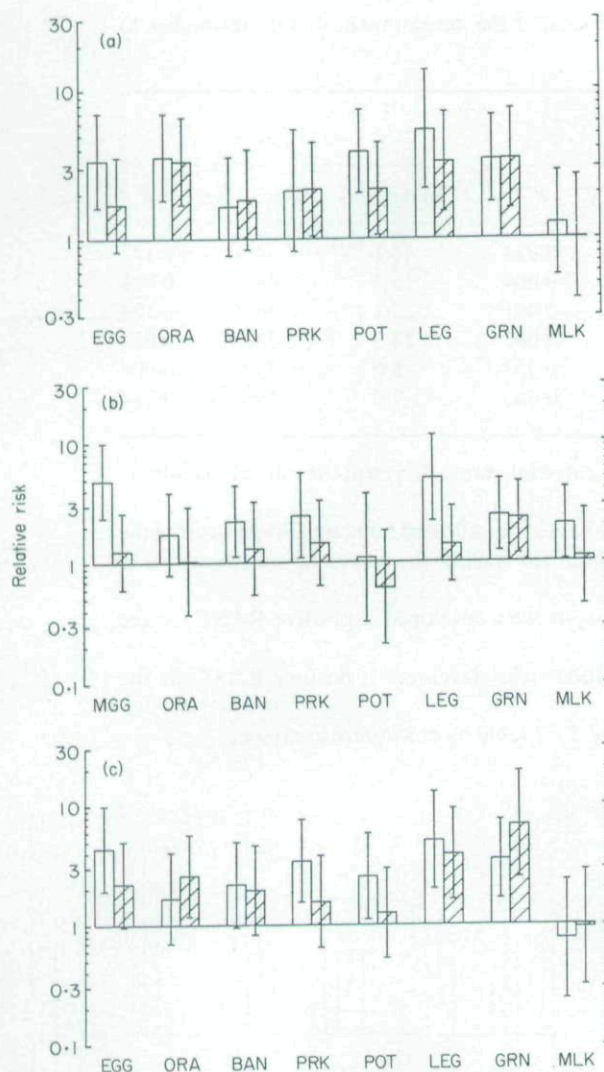


Fig. 3. IgG₁ (□) and IgG₄ (▨) antibody responses to individual foods (see Subjects and methods) predicting the development of IgE antibodies to the inhalant allergens, grass pollen (a), house dust mite (b) and cat dander (c). Results are expressed as relative risk (95% confidence interval).

conversions for cat dander and especially mite, the IgG₁ anti-food response was more informative. However, for the development of IgE antibodies to dog dander, the IgG₄ anti-food response was a better predictor than the corresponding IgG₁ response. For the prediction of grass-pollen specific IgE sero-conversions, both the IgG₁ and the IgG₄ anti-food responses were informative, the IgG₄ response being slightly better than the IgG₁ response.

Surprisingly, in the present high-risk group we did not find elevated levels of total IgG₄ more frequently in children with (a history of) atopic eczema than in children without (a history of) eczema. In addition, the IgG₁ and

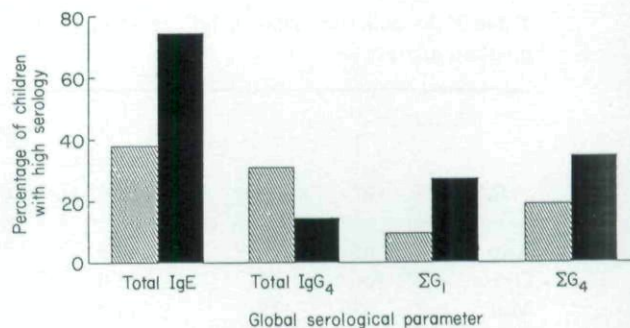


Fig. 4. Global serological parameters in relation to the presence (■) or absence (▨) of (a history of) atopic eczema. The percentage of children with high levels of total IgE or total IgG₄ (higher levels than the 75-percentiles of age-clustered unselected children; [14]) as well as the percentage of children with a positive ΣG₁ or ΣG₄ score are given.

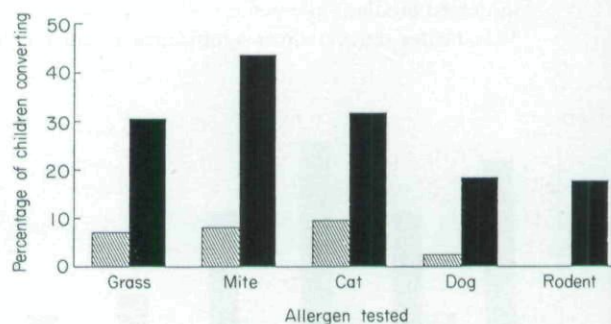


Fig. 5. IgE sero-conversion in relation to the presence (■) or absence (▨) of (a history of) atopic eczema. The percentage of children developing IgE antibodies to grass pollen, house dust mite and danders from cat, dog and rodent are given.

IgG₄ anti-food responses tended to be higher in the eczema group, but this phenomenon was statistically significant only for IgG₁ anti-food. Therefore, we conclude that, in general, the IgG₄ antibody response to foods is not a better predictor than the corresponding IgG₁ antibody response.

The effect of atopic eczema

In the present study we found a marked association between the levels of IgG₁ and also IgG₄ antibodies to foods and the development of IgE antibodies to inhalant allergens at a later time. However, the observed association might be due to the association of each of these parameters with atopic eczema.

Table 4. Association between IgG antibody response to foods and the development of IgE antibodies to inhalant allergens in children with (a history of) atopic eczema

RAST	n*	n (A1)†	IgG ₁		P**	IgG ₄		P
			ΣG ₁ -(%)‡	ΣG ₁ +(%)§		ΣG ₄ -(%)	ΣG ₄ +(%)	
Any RAST	23	3	10.0	33.3	0.842	9.5	50.0	0.599
Grass	49	15	23.7	46.2	0.286	17.6	60.0	0.009
Mite	39	17	32.3	87.5	0.016	36.5	75.0	0.107
Cat	41	13	25.0	55.6	0.182	24.1	50.0	0.211
Dog	38	7	14.3	30.0	0.532	7.1	50.0	0.012
Rodent	51	9	15.8	23.1	0.862	14.3	25.0	0.592

* Indicates the number of children with (a history of) atopic eczema and RAST negative for the indicated inhalant allergen at the start of the follow-up study.

† Indicates the number of children with (a history of) atopic eczema who developed IgE antibodies to the indicated inhalant allergen during the study, being RAST negative for the other tabulated allergens at the start of the follow-up study (members of subgroup A1).

‡ Indicates the percentage of children with (a history of) atopic eczema with low IgG₁ anti-foods who developed a positive RAST for the indicated inhalant allergen.

§ Indicates the percentage of children with (a history of) atopic eczema with high IgG₁ anti-foods who developed a positive RAST for the indicated inhalant allergen.

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

As mentioned above, in the present population the frequency of children with (a history of) atopic eczema was higher in the group who developed IgE antibodies to grass pollen, house dust mite, or danders from cat, dog and rodent than in the group who remained negative to RAST. In addition, in patients with atopic eczema an increased gut permeability was observed [24], in contrast to Bjarnason *et al.* [24], and a decreased clearance rate of ingested proteins [26]. Consequently, an elevated IgG antibody response to foods might occur.

Therefore, we analysed the association between the IgG anti-food response and the inhalant-specific IgE response by applying a statistical correction for the contribution of atopic eczema. The results indicated that the observed association was only to a small extent due to the association between IgG anti-foods and atopic eczema on the one hand and between atopic eczema and the development of IgE anti-inhalants on the other.

Crossreactivity between foods and inhalant allergens

The observed association between the IgG antibody response to foods and the development of IgE antibodies to inhalant allergens might result from a general increased permeability of mucosal membranes in atopic subjects. Such a defect in antigen exclusion might elicit an elevated antibody response to both food and inhalant antigens.

Alternatively, immunological specificity might be

involved. In our hypothesis, IgE antibodies to inhalant allergens might be generated after priming by cross-reacting antigens present in foods.

Different routes of immunological priming are known: T-cell priming and B-cell priming. In the case of T-cell priming ('carrier priming'), after preimmunization with a carrier protein, the anti-hapten immune response will be enhanced subsequent to immunization with a hapten coupled to that carrier protein [27,28]: the T-helper cells induced after pre-immunization co-operate with hapten-specific B cells. Analogously, after pre-immunization with foods T-helper cells might be generated against cross-reacting structures present in those foods. Subsequent contact with inhalant allergens containing these cross-reacting structures might result in an enhanced immune response to inhalant allergens. Because the crossreactivity is present at the T-cell level, the antibodies generated presumably do not crossreact themselves. However, those antibodies are directed against antigens that carry the crossreacting structures.

In the case of B-cell priming after pre-immunization with a carrier-coupled hapten, the anti-hapten immune response is often enhanced subsequent to immunization with the same hapten coupled to a different carrier [29]. The antibodies generated will usually react with both the primary antigen (food) and the secondary antigen (inhalant allergen).

In our hypothesis, crossreactivity between foods and

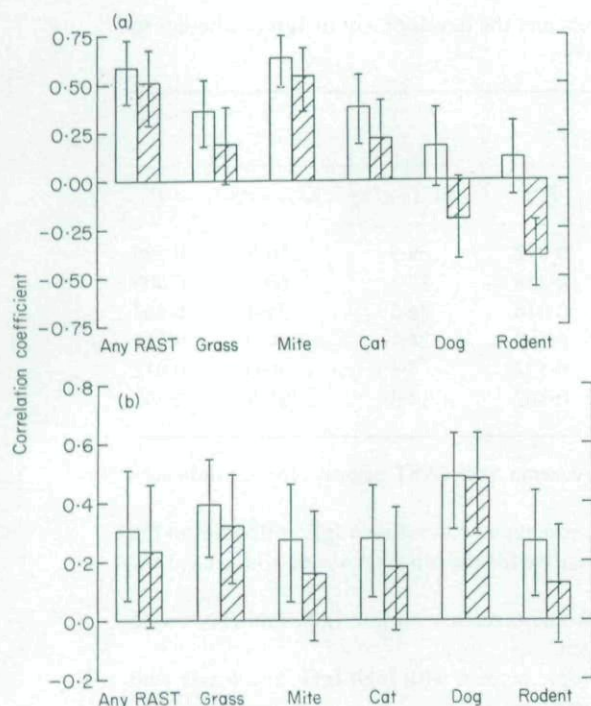


Fig. 6. Total (□) and partial (▨) correlation between the summed IgG₁ (a) or summed IgG₄ (b) antibody responses to foods and the development of IgE antibodies to inhalant allergens. Partial correlation coefficients were from calculated phi values of 2 × 2 tables [9], a statistical correlation being made for the contribution of atopic eczema to the association between the inhalant-specific IgE antibody response and the IgG antibody response to foods.

inhalant allergens plays an essential role. Therefore, the existence of such crossreactivity is of crucial importance. We, and others [12, 13, 30–34], demonstrated the presence of immunologically crossreacting structures in vegetables, fruits, pollen and even *Hymenoptera* venom.

In the active group of the present high-risk children, we also studied the specificity of the newly generated IgE antibody response to grass pollen, house dust mite and cat dander in detail. Special attention was paid to the immunological crossreactivity between foods and inhalant allergens. In early serum samples of several high-risk children, we demonstrated crossreactivity on the level of IgE antibodies, (1) between grass pollen and potato, and (2) between cat serum and components present in pork (pig serum). In both situations, the IgG antibodies to components present in these foods generally preceded the development of IgE antibodies to grass pollen and cat dander [unpublished observations]. Therefore, these results support our hypothesis involving immunological specificity: IgE antibodies to inhalants might be generated after priming by crossreacting antigens present in foods.

Acknowledgments

The authors express their gratitude to Prof. T. E. W. Feltkamp and to Dr C. Aaij, Dr T. A. Out and Dr S. O. Stapel for reviewing the manuscript. We also gratefully acknowledge all children and their parents for their willingness to participate in this study.

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

References

- Businco L, Marchetti F, Pellegrini G, Perlini R. Predictive value of cord blood IgE levels in 'at-risk' newborn babies and influence of type of feeding. *Clin Allergy* 1983; 13:503–8.
- Kaufman HS. Allergy in the newborn: skin test reactions confirmed by the Prausnitz-Kuestner test at birth. *Clin Allergy* 1971; 1:363–7.
- Kuroume T, Oguri M, Matsumura T, *et al.* Milk sensitivity and soybean sensitivity in the production of eczematous manifestations in breast-fed infants with particular reference to intra-uterine sensitization. *Ann Allergy* 1976; 37:41–6.
- Michel FB, Bousquet J, Greuillier B, Robinet-Levy M, Coulomb Y. Comparison of cord blood immunoglobulin-E concentrations and maternal allergy for the prediction of atopic disease in infancy. *J Allergy Clin Immunol* 1980; 65:422–30.
- Warner JO. Food allergy in fully breast-fed infants. *Clin Allergy* 1980; 10:133–6.
- Welsh JK, May JT. Anti-infective properties of breast milk. *J Paediatr* 1979; 94:1–9.
- Björkstén B. Does breast-feeding prevent the development of allergy? *Immunol Today* 1983; 4:215–7.
- Kramer MS. Does breast-feeding help protect against atopic disease? Biology, methodology and a golden jubilee of controversy. *J Pediatr* 1988; 112:181–90.
- Saarinén UM, Kajosaari M. Does dietary elimination in infancy prevent or only postpone a food allergy? *Lancet* 1980; i:166–7.
- Kajosaari M, Saarinén UM. Prophylaxis of atopic disease by six months total solid food elimination. *Acta Paediatr Scand* 1983; 72:411–44.
- Saarinén UM, Kajosaari M, Blackman A, Siimes MA. Prolonged breast-feeding as prophylaxis for atopic disease. *Lancet* 1977; ii:163–6.
- Aalberse RC, Koshte VL, Clemens JGJ. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen and *Hymenoptera* venom. *J Allergy Clin Immunol* 1981; 68: 356–64.
- Calkhoven PG, Aalbers M, Koshte VL, Pos O, Oei HD, Aalberse RC. Cross-reactivity among birch pollen, vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. *Allergy* 1987; 42:382–90.
- Calkhoven PG, Aalbers M, Koshte VL, Aalberse RC. Relation 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. *Clin Exp Allergy* 1991; 21:000-000.
- 15 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Dermatol Venerol* 1980; 92:44-7.
 - 16 Morris JE, Gardner MJ. Calculating confidence intervals for relative risks (odds ratios) and standardization ratios and rates. *Br J Med* 1988; 296:1313-16.
 - 17 Clarke HH, Clarke DH. Advanced statistics with applications to physical education. Englewood Cliffs: Prentice Hall, 1972; 52-8 and 115-17.
 - 18 Gwynn CM, Morrison Smith J, Leon G, Stanworth DR. IgE and IgG₄ subclass in atopic families. *Clin Allergy* 1979; 9:119-23.
 - 19 Merrett J, Barnetson RSH, Burr ML, Merrett TG. Total and specific IgG₄ antibody levels in atopic eczema. *Clin Exp Immunol* 1984; 56:645-52.
 - 20 Shakib F, McLaughlan P, Stanworth DR, Smith E, Fairburn E. Elevated serum IgE and IgG₄ in patients with atopic dermatitis. *Br J Dermatol* 1977; 97:59-63.
 - 21 Homburger HA, Mauer K, Sachs MI, O'Connell EJ, Jacob GL, Caron J. 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.
 - 22 Björkstén B, Ahlstedt S, Björkstén F, *et al.* Immunoglobulin E and immunoglobulin G₄ antibodies to cow's milk in children with cow's milk allergy. *Allergy* 1983; 38:119-24.
 - 23 Shakib F, Brown HM, Stanworth DR. Relevance of milk- and egg-specific IgG₄ in atopic eczema. *Int Arch Allergy Appl Immunol* 1984; 75:107-12.
 - 24 Jackson PG, Lessof MH, Baker RWR, Ferrett J, McDonald DM. Intestinal permeability in patients with eczema and food allergy. *Lancet* 1981; i:1285-6.
 - 25 Bjarnason I, Goolamali SK, Levi AJ, Peters TJ. Intestinal permeability in patients with atopic eczema. *Br J Dermatol* 1985; 12:291-7.
 - 26 Paganelli R, Levinsky RJ, Brostoff Wraith DG. Immune complexes containing food proteins in normal and atopic subjects after oral challenge and effect of sodium cromoglycate on antigen absorption. *Lancet* 1979; i:1270-1.
 - 27 Paul WE, Katz DH, Goidl EA, Benacerraf B. Carrier function in anti-hapten immune responses. II Specific properties of carrier cells capable of enhancing anti-hapten antibody responses. *J Exp Med* 1970; 132:283-99.
 - 28 Zöller M, Andrighetto G. Regulation of carrier-specific response via a haptenic epitope. *Scand J Immunol* 1987; 26:277-86.
 - 29 Koch G, Osmond DG, Julius MH, Benner R. The mechanism of thymus-dependent antibody formation in bone marrow. *J Immunol* 1981; 126:1447-51.
 - 30 Björkstén F, Halmepuro L, Hannuksela M, Lahti A. Extraction and properties of apple allergens. *Allergy* 1980; 35:671-7.
 - 31 De Martino M, Novembre E, Cozza G, De Marco A, Bonazza P, Vierrucci A. Sensitivity to tomato and peanut allergens in children monosensitized to grass pollen. *Allergy* 1988; 43:206-13.
 - 32 Halmepuro L, Lowenstein H. Immunological investigation of possible structural similarities between pollen antigens and antigens in apple, carrot and celery tuber. *Allergy* 1985; 40:264-72.
 - 33 Halmepuro L, Vuontela K, Kalimo K, Björkstén F. Cross-reactivity of IgE antibodies with allergens in birch pollen, fruits and vegetables. *Int Arch Allergy Appl Immunol* 1984; 74:235-40.
 - 34 Lahti A, Björkstén F, Hannuksela M. Allergy to birch pollen and apple, and cross-reactivity of the allergens studied with the RAST. *Allergy* 1980; 35:297-300.

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.