

Clinical and Immunological Studies in the Parents Whose Children Showed Severe IgE-Mediated Food Allergies

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ABSTRACT

Our objective was to analyze the clinical and immunological responses to food within two generations. The parental histories of atopic diseases, serum total IgE and specific IgE to food, and maternal specific IgG to egg-white and milk, and total secretory-IgA (sIgA) in saliva were examined in 18 nuclear families including children with severe IgE-mediated food allergies. The results were then compared with those for 47 males and 69 females, age-matched, whose children were free of atopic diseases. When analyzed statistically, the following were found to be significantly different between the two groups. (1) The frequency of a maternal history of an adverse reactions to food in the food-allergy group was higher than that in the nonallergy group (3/18 vs 1/69, $p < 0.05$), however, the maternal specific sensitization to the foods did not correspond to that in the children. (2) The frequency of maternal total IgE levels of more than 400 IU/ml in the food-allergy group was higher than that in the nonallergy group (6/18 vs 7/69, $p < 0.05$). The maternal levels of specific IgG to the foods and total sIgA were not statistically different between the two groups. The maternal atopic status as to food seems to be more decisive than that the paternal one with regard to a child's risk of food allergy, but the biological contact between child and mother *in utero* does not seem to play a role in the development of food allergy.

INTRODUCTION

ALTHOUGH THE PREVALENCE of IgE-mediated food allergy in childhood is unknown, there are some reports in the medical literature of fatal or near-fatal anaphylactic reactions caused by food.⁽¹⁾ Recently, Holgate and co-workers⁽²⁾ suggested that an anaphylactic reaction to food is one explanation for sudden unexplained postperinatal death. Hereditary and environmental factors are the most important causes of allergies, and several studies indicated that maternal dietary elimination during pregnancy may protect a baby against allergy.^(3,4) Therefore, when a child has suffered from severe IgE-mediated food allergy, some pediatric allergists may advise the mother to avoid the offending food during following pregnancies. However, no reports have appeared including population-based data on the specific immunologic responses to food within family groups. This seems surprising since recent immunogenetic studies indicated such antigen specificity of inheritance exists.^(5,6) Furthermore, various humoral maternal and external factors may pass through placenta and influence *in utero* the IgE-producing system of the fetus.^(7,8) To investigate the relationship be-

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tween parents and their children, we analyzed the clinical and immunological responses to food within two generations.

MATERIALS AND METHODS

Patients and controls

Nineteen patients with severe IgE-mediated food allergy in 18 families were selected from the patient population seen at the Allergy Clinic of the Department of Child Development, Kumamoto University Hospital, Kumamoto (food-allergy group). Each has a history of a systemic reaction to food (egg, 11 cases; milk, 2 cases; egg + milk, 4 cases; egg + milk + wheat, 1 case; egg + milk + wheat + buckwheat, 1 case), e.g., systemic hives or angioedema with wheezing or hypotension within 30 min following ingestion of the food. An adverse food reaction of a nonimmunological cause may mimic a food allergy, and the skin prick test and specific IgE antibody measurement in serum have limited reliability for diagnosis. In addition, an oral challenge with the offending food is supposed to cause a serious reaction in such cases. Since an anaphylactic reaction to food was closely associated with positive oral Prausnitz-Küstner (oP-K) test in our previous experiment,⁽⁹⁾ the food-allergy group in this study was composed of patients who showed a positive oP-K reaction to food. The characteristics of the patients are summarized in Table 1. Forty-seven males and 69 females served as controls in this study, and their children did not suffer from any allergic diseases (nonallergy group). They comprised a random population taken from the medical staff or blood donation volunteers. All females in this study were free from gastrointestinal diseases or active dental caries.

A past or present history of atopy, such as asthma, allergic rhinitis/conjunctivitis, or atopic eczema, or an adverse reaction to food in these subjects was ascertained by means of a careful interview, present findings, or examination of medical records.

oP-K test

The oP-K reaction was described originally in 1926 by Walzer,⁽¹⁰⁾ and was reintroduced recently by Aoki et al.⁽¹¹⁾ Before the test in this study, informed consent was obtained from the parents. The recipients were limited to the patients' mothers, who had shown no allergy to the relevant food and were well aware of the medical history of the patients. All patients had not received a blood transfusion and had no history of hepatitis. Their blood test results as to hepatitis B, hepatitis C, and HTLV-I were all negative. From 2 days prior to the passive serum transfer, all recipients were prevented from taking the offending food. One hundred microliter of patient's serum was injected intracutaneously into the forearm of the patient's mother. Heat (56°C, 30 min)-treated serum was also injected into the forearm on the other side. Two days after the inoculation, a food allergen (a raw hen's egg, 200 ml of milk, or 20 g of melted wheat or buckwheat) was given to the mother. The reaction manifested itself at the sensitized site, first as a faint blush and then as a gradually increasing erythema, accompanied or at times preceded by severe itching. Tiny wheals then appear, which grew and fused until a large central wheal resulted. No reaction was observed on the skin where heat-inactivated serum was inoculated.

IgE assay

Total serum IgE levels were measured using the Phadebas paper radioimmunosorbent test (Pharmacia A.B., Uppsala, Sweden) in international units per milliliter. Serum-specific IgE to food was titrated by means of the fluoroenzyme immunoassay using a CAP-RAST Kit (Pharmacia A.B., Uppsala, Sweden). The results showing more than class 2 were considered as being positive.

Assays for specific IgG and total sIgA

The levels of specific IgG to hen's egg-white and cow's milk in serum, and total sIgA in saliva were measured by means of an enzyme-linked immunosorbent assay (ELISA). For the specific IgG assay,⁽¹²⁾ microtiter plates (Imulon 2; Dynatech Lab., Alexandria, VA) were initially coated with 5 µg/ml of diluted standardized food allergens (Torii, Tokyo). After blocking with 1% bovine serum albumin, 1:100 to 1:1,000

PARENTAL FACTORS IN FOOD ALLERGIES

TABLE 1. CLINICAL AND LABORATORY DATA ON PATIENTS WITH SEVERE IGE-MEDIATED FOOD ALLERGIES

Case	Age/sex	Total IgE (IU/ml)	Offending food (CAP-RAST classes)	Complications ^a
1.	10M/M	13	Egg(1)	
2.	7Y/M	872	Egg(3) Milk(5)	AD, BA
3.	15M/M	19	Egg(3)	
4.	8M/F	59	Egg(3)	
5.	5M/M	114	Egg(2)	AD
6.	2Y/M	797	Egg(4)	
7.	11M/F	17	Egg(2)	
8.	3Y/M	607	Egg(3) Milk(2)	
9.	2Y/M	437	Milk(4)	
10.	5Y/F	145	Egg(1)	
11.	4Y/M	712	Egg(4)	AD, BA
12.	9M/F	43	Egg(3)	
13.	14M/M	2,950	Egg(5)	AD, BA
14.	10M/F	39	Egg(4)	
15.	14M/M	351	Egg(4) Milk(4) Wheat(4) Buckwheat(1)	
16.	17M/F	128	Egg(2) Milk(3) Wheat(3)	
17.	7M/F	8	Milk(3)	
18.	5Y/M	5,280	Egg(3) Milk(5)	AD
19.	3Y/F	547	Egg(3) Milk(5) Wheat(4)	

^aAD, atopic dermatitis; BA, bronchial asthma.

diluted serum samples were added. The unbound samples were decanted, and the plates were then incubated with peroxidase-labeled, 1:10,000 diluted antihuman IgG antibody (Kirkegaard & Perry Labs, Gaithersburg, MD). After washing, the plates were incubated with *o*-phenylenediamine containing H₂O₂, and the reaction was quenched with H₂SO₄. The absorbance values were read at OD 492 nm. A standard curve for specific IgG was obtained by using pooled serum from normal volunteers. The OD value of 400-times diluted pooled serum was determined to be 100 units for hen's egg-white, and the OD value of 100-times diluted serum was calculated to be 100 units for cow's milk. The minimum sensitivity of both assays was 12.5 units.

An EIA S-IgA Test Kit (MBL Igaku-Seibutsu, Nagoya) was used for the sIgA assay according to the manufacturer's instructions. This kit includes an antihuman secretory component as the first antibody and peroxidase-labeled antihuman IgA as the second antibody. A standard curve for sIgA was obtained by using affinity-purified sIgA from human colostrum, and the results were expressed as μ g/ml of saliva.

Data analysis

The data for IgE and specific IgG were logarithmically transformed before statistical analysis. Group means were compared by means of Student's *t* test. Frequencies were compared as to their statistical significance with the chi-square test. When the frequency was less than 5 in the analysis, Fisher's exact test was used. *p* values of less than 0.05 were considered significant.

TABLE 2. PARENTAL HISTORIES OF ATOPIC DISEASES IN THE FOOD-ALLERGY AND NONALLERGY GROUPS

	<i>Food-allergy group</i>	<i>Nonallergy group</i>
Parental history of asthma, eczema, rhinitis, and/or conjunctivitis		
Fathers	6/18	8/47
Mothers	0/18	11/69
Parental history of IgE-mediated adverse food reactions		
Fathers	1/18	0/47
Mothers	3/18 ^a	1/69 ^a

^aStatistically significant ($p < 0.05$).

RESULTS

The parental histories of atopic diseases are summarized in Table 2. The frequency of fathers who suffered from atopic diseases, such as bronchial asthma, atopic eczema, or allergic rhinitis/conjunctivitis, in the food allergy group was not statistically different from that in the nonallergy group. Although all the mothers in the food allergy group did not have any respiratory or skin allergies, this was not statistically significant when compared with those in the nonallergy group ($p = 0.065$).

One father and three mothers in the food-allergy group had histories of adverse reactions to foods (generalized urticaria to shrimp/crab in the father, and oral itching and lip angioedema to buckwheat, generalized urticaria to cheese/mackerel, and oral itching to melon in the mothers, respectively). On the contrary, none of the 47 fathers and only 1 of the 69 mothers (dermal itching to egg) showed adverse reactions to food in the nonallergy group. Although the 95% confidence intervals by Fisher's exact test is somewhat wide (1.1–88.6), the frequency of mothers who suffered from adverse reactions to food in the food-allergy group was significantly higher than that in the nonallergy group. All the parents with adverse food reactions in this study showed positive CAP-RAST results or positive skin prick test results, but the foods causing adverse reactions in these parents did not correspond to those causing severe allergies in their children.

The serum-IgE levels are shown in Table 3. The geometric means between the parents in both groups were not statistically different. However, the frequency of a level of more than 400 IU/ml of IgE in the mothers in the food-allergy group was significantly higher than that in the nonallergy group ($p < 0.05$).

When we determined the parental sensitization to the foods that caused systemic reactions in the children, two mothers in the food-allergy group were found to show positive CAP-RAST results as to the offending foods in their children. However, these mothers have not shown any adverse reactions to the foods.

TABLE 3. THE SERUM TOTAL-IGE LEVELS IN THE PARENTS IN THE FOOD-ALLERGY AND NONALLERGY GROUPS

	<i>Food-allergy group</i>	<i>Nonallergy group</i>
The geometric means and 1 SD ranges of total IgE levels (IU/ml)		
Fathers	100, 35–288	91, 19–241
Mothers	110, 23–513	97, 29–307
The subjects showing more than 400 IU/ml of total IgE		
Fathers	3/18	5/47
Mothers	6/18 ^a	7/69 ^a

^aStatistically significant ($p < 0.05$).

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When CAP-RAST was examined as to egg, milk, wheat, and buckwheat in the parents from nonallergy group, one mother who had a history of an adverse reaction to egg showed a positive result as to egg, and one more mother, who has not shown any adverse reaction to buckwheat, had a positive result as to buckwheat. Therefore, the maternal frequencies of sensitization to the offending foods in the food-allergy group were not statistically different when compared with those in the nonallergy group. None of the fathers in either group showed positive CAP-RAST results as to the offending foods.

When serum-specific IgG as to egg-white or milk was examined, more than half of the mothers from both food-allergy and nonallergy groups showed results under the sensitivities of both ELISAs in this study. The frequencies of subjects showing results under the sensitivities of IgG assays for egg-white and milk in the mothers in the food-allergy group were not statistically different from those in the nonallergy group (egg, 10/18 vs 41/69; milk, 13/18 vs 49/69). In addition, both IgG values in the subjects showing results over the sensitivities of the ELISAs were not statistically different between the mothers in the food-allergy and nonallergy groups (egg, 63, 20–200 vs 40, 10–158; milk, 35, 16–63 vs 32, 13–79, geometric means and 1 standard deviation ranges, respectively). The means and 1 standard deviations of sIgA levels in saliva of the mothers in the food-allergy group and those in the nonallergy group were 203 ± 93 $\mu\text{g/ml}$ and 235 ± 111 g/ml , respectively. These values were not statistically different.

DISCUSSION

Study on genetic restriction of food-specific IgE responses is important clinically and for understanding the control of dietary cures in the field of food allergies. However, no reports have appeared including population-based data on the specific immune responses to food allergens among family members. Although the present data did not show any correlation between parental sensitization to food allergens and an outbreak of adverse food reactions in the child, disease-specific inheritance seems to be involved in food allergies. In addition, the influence of the mother's status is more decisive as to the genetic risk than that of the father. Recently, Cookson and co-workers⁽¹³⁾ showed that the gene locus for IgE-mediated allergies was assigned to chromosome 11, and suggested that transmission of this "atopic gene" at the chromosome 11 locus is detectable only through the maternal line. Therefore, it is possible that the result of present study is in accord with the observation by Cookson and co-workers.

Several previous studies indicated that maternal atopy in general increases the risk of atopy in children.^(14,15) The most convincing of these were large studies on newborn infants, which revealed that cord-blood IgE concentrations were significantly higher in the children of atopic mothers than those of atopic fathers.⁽⁸⁾ Another hypothesis is that there is a higher environmental congruence between mother and child than father and child. Intrauterine events occurring in mothers could be one explanation. The biological contact with the child's immune system by this means is of course lacking in fathers. In rodents, maternal IgG has been shown to modify the subsequent development of the infant IgE responses,⁽⁷⁾ and sIgA in the digestive tract is highly involved in the host defense against the penetration of food.⁽¹⁶⁾ However, the maternal levels of serum specific IgG to food and total sIgA in saliva were not statistically different between the food-allergy and nonallergy groups in this study. Therefore, the results did not support the hypothesis that the biological contact between child and mother *in utero* may play a role in food allergies.

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