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Antibody responses in watermelon sensitivity

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*Watermelon-specific IgE (WM-IgE) appears to mediate the oropharyngeal symptoms after watermelon ingestion. However, less than one fourth of watermelon-sensitive individuals (i.e., those with detectable WM-IgE) develop such symptoms after watermelon ingestion. In an attempt to separate symptomatic from asymptomatic persons, we measured watermelon-specific-IgE and IgG₄ concentrations in the sera of 29 watermelon-sensitive patients, 6 of whom were symptomatic. Although the mean WM-IgE level of the symptomatic group exceeded that of the asymptomatic group (p = 0.04), individual WM-IgE values failed to distinguish symptomatic from asymptomatic persons. No differences in WM-IgG₄ levels between the two groups could be found. Watermelon extract proteins were separated by isoelectric focusing and blotted to nitrocellulose membranes. The membranes were reacted with the individual sera of 14 watermelon-sensitive patients and then stained for IgE, IgG₁, and IgG₄ binding. Great variations in IgE, IgG₁, and IgG₄ binding patterns were found, but no subtype restrictions were discovered. Although the pattern of IgE-stained bands in the absence of corresponding IgG₁-stained bands was seen more frequently in symptomatic individuals, immunoblot patterns failed to separate symptomatic from asymptomatic persons. We conclude that the pattern of IgE, IgG₁, and IgG₄ responses to separated watermelon allergens was not predictive of clinical symptoms in a group of watermelon-sensitive patients. (*J ALLERGY CLIN IMMUNOL* 1988;82:795-800.)*

Practicing allergists commonly see individuals who describe the development of itching and/or swelling of the lips, tongue, or throat shortly after food ingestion. We have previously reported an association between oropharyngeal symptoms after the ingestion of

Abbreviations used

WM-IgE:	Watermelon-specific IgE
WM-IgG ₁ :	Watermelon-specific IgG, subclass 1
WM-IgG ₄ :	Watermelon-specific IgG, subclass 4
RW-IgE:	Ragweed-specific IgE
PBS:	0.05 mol/L phosphate and 0.1 mol/L NaCl at pH 7.5 with sodium azide 0.1%
FCS-PBS:	Fetal calf serum diluted with 0.05 mol/L phosphate and 0.1 mol/L NaCl at pH 7.5 with sodium azide 0.1%
BSA-PBS:	Bovine serum albumin diluted with 0.05 mol/L phosphate and 0.1 mol/L NaCl at pH 7.5 without azide

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TABLE I. Characteristics of symptomatic and asymptomatic patients

	Symptomatic (n = 6)	Asymptomatic (n = 23)	Significance (<i>p</i> value)
Age (yr)	34 (14-67)	31 (10-58)	NS
Male:female	6:0	11:12	0.02
Serum IgE* (U/ml)	787	608	NS
WM-IgE	9.1 ± 5.6	5.3 ± 3.2	0.04
WM-IgG ₄	6.0 ± 6.9	11.9 ± 11.0	NS
RW-IgE	35.9 ± 7.3	25.5 ± 12.5	NS

NS = Not significant.

Data are the mean ± SD (range).

*Geometric mean.

watermelon or other gourd family members and the presence of gourd-specific IgE in the individual's serum.¹ Although specific IgE was found in the serum of almost all of those who developed oropharyngeal symptoms, less than one fourth of patients with detectable specific IgE reported symptoms. Thus, oropharyngeal symptoms after gourd ingestion cannot be accurately predicted by the detection of gourd-specific IgE. The purpose of this study was to determine whether oropharyngeal symptoms after watermelon ingestion could be predicted by the measurement of watermelon-specific IgE and IgG₄ or by the detection of IgE, IgG₁, and IgG₄ directed against particular individual watermelon antigens. We also wished to learn if the immune responses to individual allergens would be restricted to certain IgG subclasses. IgG subtype restriction of immune response has been reported for some antigens^{2,3} and questions concerning the role of immunoglobulin subclasses in allergy have been raised.⁴⁻⁷

METHODS

Patients

Serum samples from 29 patients previously seen in the Henry Ford Hospital allergy clinics were chosen for study. Each sample contained at least 180 IU/ml of IgE as measured by ELISA,⁸ had detectable concentrations of WM-IgE, and was contributed by an individual available for telephone interview. Although previous work¹ has shown that a WM-IgE concentration ≥ 1.8 may be considered positive, the lowest concentration of WM-IgE in the present group of patients was 2.2. Serum samples were chosen without regard to the patients' clinical status.

During telephone interviews, each subject was asked whether he or she had food allergies and then specifically asked about oropharyngeal symptoms after watermelon ingestion. Food histories were considered positive only if symptoms began within 30 minutes of food ingestion and only if the subject experienced the same symptoms on at least two occasions within the past 2 years. Subjects were

interviewed in random order by two of the authors, who used a standard questionnaire and who were blinded as to the subjects' laboratory values.

Extracts

Watermelon extract was made from fresh, locally obtained watermelon and contained 1.4 mg of protein/ml.⁹ The short ragweed extract (Hollister-Stier, Spokane, Wash.: 1:20, 50% glycerine, lot D5156M) contained 8.4 mg of protein/ml.⁹

ELISA

Concentrations of WM-IgE and RW-IgE were measured with the biotin-avidin amplified ELISA described previously.⁸ Briefly, the watermelon extract was diluted 1:100 with 60 mmol/L carbonate/bicarbonate, pH 7 buffer, and adsorbed to plastic microwells overnight. Ragweed extract diluted 1:250 was similarly adsorbed. Sequentially, with interspersed washes, a study serum, biotin-rabbit antihuman IgE conjugate, avidin-alkaline phosphatase conjugate, diethanolamine buffer, and 4-methylumbelliferyl phosphate were added. Fluorescence proportional to IgE bound to watermelon or ragweed was measured by a microfluorimeter. The values reported for each of these assays are the ratio of the mean of duplicate determinations for a study serum compared with the mean of duplicate determinations for negative pooled sera.

The same assay, with modifications, was used to detect WM-IgG₄. To each well previously coated with watermelon extract, 50 μ l of the study serum was added. After overnight incubation at room temperature and washing, murin monoclonal antihuman IgG₄ (Unipath Limited, Bedford, England; clone RJ4), diluted 1:1000 with 1% FCS-PBS, was added. The wells were incubated for 2 hours at 37° C and washed thrice. Then 100 μ l of biotin goat antimouse conjugate, diluted 1:500 with 1% FCS-PBS, was added, followed by incubation for 2 hours at 37° C. The remaining procedures in the assay were identical to those of the WM-IgE assay. The values reported for the WM-IgG₄ assays are the ratio of the mean of duplicate determinations for a study serum compared with the mean of duplicate determinations for wells to which only fetal calf serum had been added.

TABLE II. Characteristics of male and female patients

	Men (n = 17)	Women (n = 12)	Significance (p value)
Age (yr)	31 (14-67)	33 (10-52)	NS
Symptomatic: asymptomatic	6:11	0:12	0.02
Serum IgE* (U/ml)	761	504	NS
WM-IgE	7.6 ± 4.6	3.9 ± 1.5	0.01
WM-IgG ₄	10.1 ± 11.0	11.5 ± 10.1	NS
RW-IgE	32.9 ± 9.8	20.3 ± 12.0	0.004

NS = Not significant.

Data are the mean ± SD (range).

*Geometric mean.

Isoelectric focusing and transfer to nitrocellulose

Watermelon extract was subjected to electrofocusing in 2% polyacrylamide gel in the presence of Ampholine (LKB, Gaithersburg, Md.) at a pH range between 4 and 6 as previously described.^{1, 10} Electrofocusing was performed at 4° C and 8 W constant power for 3 hours. This procedure separates the various watermelon proteins on the basis of the individual proteins' isoelectric points. In previous work, at least 15 protein bands resulted from the isoelectric focusing of watermelon extract, and most of these focused within the pH range of 4 to 6.¹ After electrofocusing, the separated watermelon proteins were electrophoretically transferred to nitrocellulose membranes (Bio-Rad, Richmond, Calif.).^{11, 12} The electrophoretic conditions were 0.7% acetic acid buffer, pH 2.5, at 70 V for 5 hours with the use of a Transblot cell (Bio-Rad).

Detection of IgE, IgG₁, and IgG₄ binding in the separated proteins

The nitrocellulose membranes were blocked for 4 hours with 10% BSA-PBS and then incubated 16 hours either with an individual study serum or with pooled sera known to contain WM-IgE. The serum pool was obtained by mixing 20 individual serum samples, each containing WM-IgG at more than fivefold the concentration in negative control sera. Each membrane was incubated with 200 µl of sera diluted with 1800 µl of PBS. In the assay used to detect IgE, the nitrocellulose membranes were incubated while agitated for 4 hours at room temperature with rabbit antihuman IgE (Calbiochem-Behring, La Jolla, Calif.) diluted 1:150 in 1% BSA-PBS. IgE bound to proteins was then identified with the Immuno-Blot assay (Bio-Rad), in which the membranes were incubated 1 hour with a goat antirabbit horseradish peroxidase conjugate diluted 1:500 with 1% BSA-PBS, washed, and covered with a color development reagent.¹³

Detection of IgG₁ and IgG₄ was similar to the procedure just described and utilized murine monoclonal antihuman IgG₁ and IgG₄ (Unipath Limited; clones NL16 and RJ4) diluted 1:500 with 1% BSA-PBS. In these assays, the nitrocellulose membranes were incubated with one of the

monoclonal antibodies for 2 hours at 37° C while agitated gently. The membranes were then incubated 2 hours at 37° C with a goat antimouse horseradish peroxidase conjugate (Bio-Rad; lot 30468) diluted 1:500 with 1% BSA-PBS, washed, and covered with a color development reagent.

Statistics

The data were analyzed by the Michigan Interactive Data Analysis System (MIDAS).¹⁴ For comparison of group means, the parametric *t* test was computed. The significance of differences between the proportion of men in the groups was determined by use of the chi-square statistic.

RESULTS

Six of 26 subjects reported developing oropharyngeal symptoms after watermelon ingestion. As indicated in Table I, symptomatic and asymptomatic individuals were similar with respect to age and serum IgE concentrations. Approximately half the asymptomatic individuals were men; in contrast, all the symptomatic individuals were men (*p* = 0.02). The WM-IgE level was significantly greater in symptomatic than in asymptomatic persons; however, no significant difference in WM-IgG₄ level was found between the symptomatic and the asymptomatic patients. Because of the striking male preponderance in symptomatic individuals, the data were retabulated on the basis of sex (Table II). Although no differences in age, total IgE levels, or WM-IgG₄ levels were demonstrated, men had significantly higher serum concentrations of WM-IgE and RW-IgE than did women.

Based on serum availability, 14 of the 29 serum samples were chosen for further study. Five of the 14 individuals contributing these sera were symptomatic. Shown in Fig. 1 are three pairs of watermelon immunoblots reacted with pooled sera and stained in duplicate for IgE, IgG₁, and IgG₄. A major, densely stained band can be seen toward the top of all of the

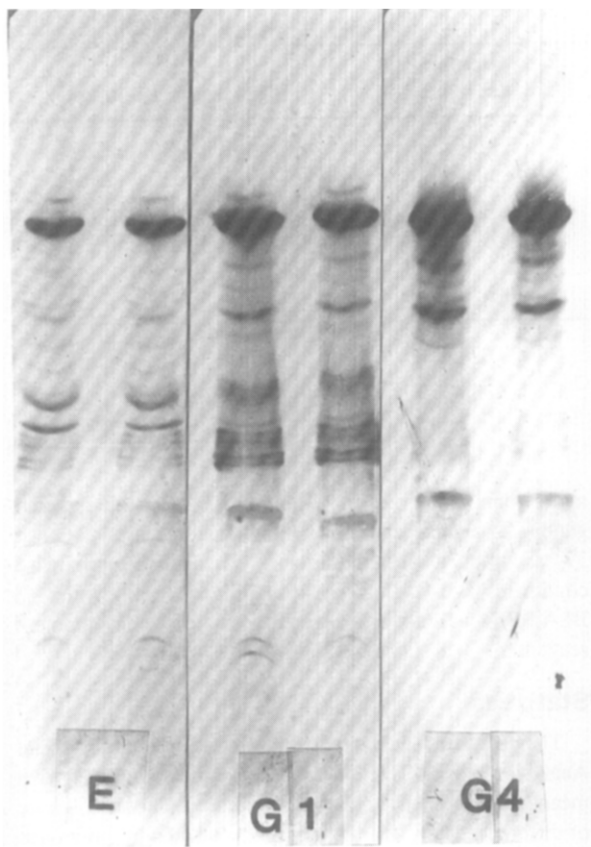


Fig. 1. Immunoblots resulting from isoelectric focusing of watermelon proteins reacted with pooled sera and stained in duplicate for IgE, IgG₁, and IgG₄ binding. A dominant antigen may be seen toward the top of each immunoblot. The anode, or area of lowest pH, is at the top of each immunoblot.

immunoblots. Although there were differences in staining intensity, all the separated watermelon proteins to which IgE bound also bound with IgG₁. In contrast, many of the protein bands were not easily detected when stained for IgG₄.

Each of the 14 serum samples were individually reacted with three watermelon blots, which were then stained for IgE, IgG₁, and IgG₄ binding to the separated watermelon proteins. Thus, each patient had a blot stained for IgE, for IgG₁, and for IgG₄. Immunoblots of the five symptomatic patients and the nine asymptomatic patients are paired in Figs. 2, A and B, respectively. In each instance the blot on the left was stained for IgE binding and the blot on the right was stained for IgG₁ binding. There was great variation in the IgE binding patterns, indicating that a given protein was not allergenic in all individuals. No pattern distinguished symptomatic from asymptomatic individuals. The allergenicity of any given watermelon

protein or combination of proteins did not predict oropharyngeal symptoms. There was also great variation in the IgG₁ binding patterns, but the patterns of symptomatic patients could not be differentiated from asymptomatic patients. Few bands were seen on the immunoblots stained for IgG₄ binding (these immunoblots not shown). Again, there was great variation in the IgG₄ binding patterns, and no pattern distinguished the symptomatic from the asymptomatic individuals.

With respect to symptomatic patient K, two bands darkly stained for IgE were not stained for IgG₁. Patient J also had bands stained for IgE without corresponding IgG₁-stained bands. This combination of IgE-stained bands in the absence of corresponding IgG₁ stained bands was seen in only one of the asymptomatic patients (patient G). With respect to the remaining eight asymptomatic patients, virtually all of the bands stained for IgE were also stained for IgG₁. This relationship was also true, however, for three of the five symptomatic patients. The major band identified in the blots that reacted with pooled sera was seen in all five symptomatic patients but in only four of nine asymptomatic patients. When the immunoblot sets of individual patients were considered, no patterns involving IgG₄ bands emerged.

DISCUSSION

An association between symptoms after food ingestion and specific IgE levels has been shown for a variety of foods. Only a minority of individuals with food-specific IgE, however, become symptomatic after ingesting the food to which their IgE is directed. With the use of watermelon as a study food, we wished to determine whether the combination of specific IgE and IgG₄ concentrations or the presence of IgE, IgG₁, or IgG₄ directed against individual food proteins would predict symptoms after food ingestion.

Concentrations of WM-IgE were higher in the symptomatic than in the asymptomatic group. Because of the variation among individual values, however, WM-IgE failed to predict an individual's symptoms. Unexpected was the finding that all symptomatic patients were men. No differences in age, total IgE level, or WM-IgG₄ level were found between men and women. In contrast, levels of both RW-IgE and WM-IgE, which have been shown to cross-react,¹ were higher in men. The reason for these differences is not known; it may be a sampling error related to the small number of subjects.

Although IgG₄ has been shown to be homocytotropic,⁵⁻⁷ its role in food allergy remains obscure. We could not detect differences in WM-IgG₄ concentra-

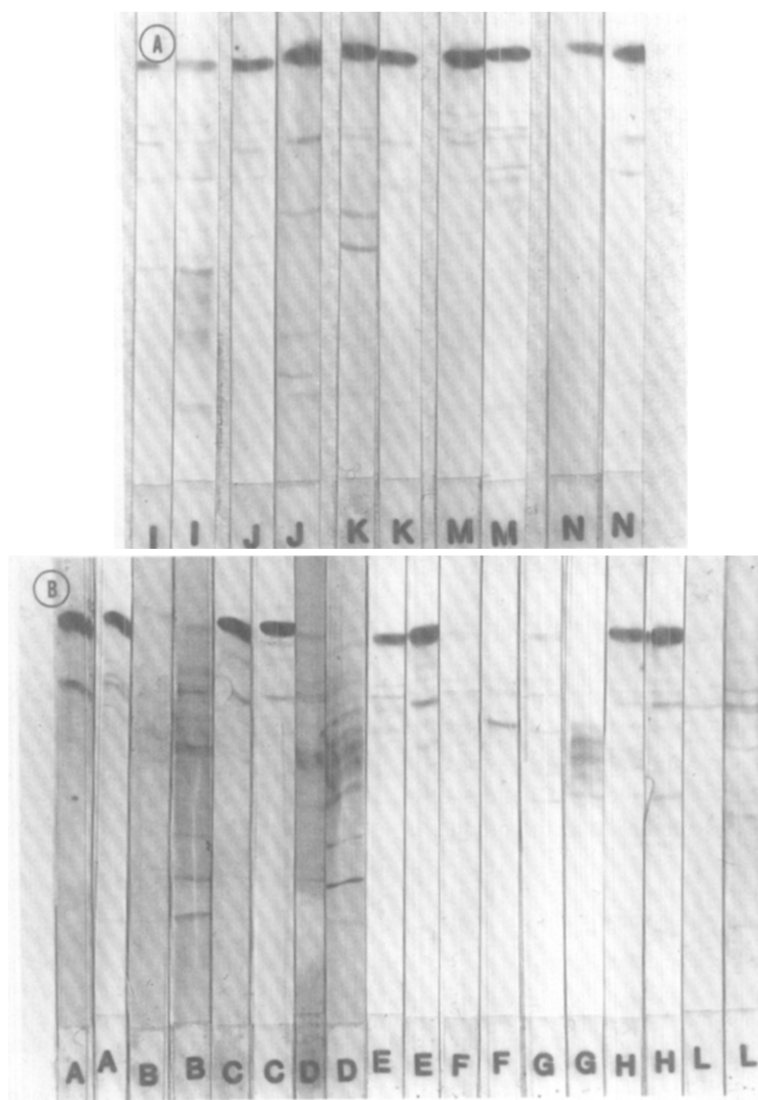


Fig. 2. Pairs of immunoblots resulting from isoelectric focusing of watermelon proteins reacted with individual serum samples from (A) symptomatic and (B) asymptomatic patients. In each pair the blot on the left is stained for IgE binding, while the blot on the right is stained for IgG₁ binding. The anode, or area of lowest pH, is at the top of each immunoblot.

tions between symptomatic and asymptomatic patients. Our findings are consistent with those of Nakagawa et al.,¹⁵ who were not able to show any clinical relevance of egg white-specific IgG₄. Furthermore, egg- and milk-specific IgG₄ have been observed frequently in a community survey of normal adults.¹⁶ Several authors, it should be noted, have reported symptomatic, food-allergic individuals whose sera contains food-specific IgG₄ in the absence of specific IgE.^{15, 17, 18}

After transfer to nitrocellulose, the separated watermelon proteins were reacted with pooled sera and stained for IgE, IgG₁, or IgG₄ binding. Presumably,

the pool identified all of the antigens recognized by each immunoglobulin isotype. Individual watermelon immunoblots were stained to identify allergen-specific IgE, IgG₁, and IgG₄ for each individual. Each immunoblot represents a profile of an individual's isotype or subtype response to a series of proteins.

On reaction with pooled sera, each band stained for IgE also stained for IgG₁, indicating that each of the stained watermelon extract proteins is capable of evoking both an IgE and an IgG₁ response. When confronted with these potentially allergenic watermelon proteins, the individual's response differs from that of the group. In most individuals, only a few proteins

elicit an IgE response. Furthermore, different proteins are allergenic in different individuals, and no single protein was found to be allergenic for all patients. The variable IgE response to watermelon proteins is similar to that found in *Dermatophagoides pteronyssinus* and orchard grass sensitivity.^{19, 20} Our patients had similar variability in their IgG₁ response to watermelon proteins. Individuals with IgE-stained bands in the absence of corresponding IgG₁-stained bands demonstrate that a given protein that evokes a response from one isotype does not necessarily evoke a response from all isotypes. In fact, IgE, IgG₁, and IgG₄ responses to watermelon, a complex mixture of antigens, often differ within a single individual. No isotype restriction could be found. The pattern of IgE-stained bands in the absence of corresponding IgG₁-stained bands appears to occur more frequently in symptomatic patients, suggesting that specific IgG₁ may be protective in some cases.

Neither serum concentrations of WM-IgE and WM-IgG₄ nor the patterns of IgE, IgG₁, and IgG₄ response or lack thereof differentiates symptomatic from asymptomatic individuals. Although the oropharyngeal symptoms after watermelon ingestion appear to be IgE mediated,¹ factors other than the pattern of antibody responses must be important in the production of symptoms.

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