

Rice hypersensitivity associated with serum complement depression

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Summary

We have studied an infant who had flushing, vomiting and diarrhoea after the first and seven subsequent feedings of rice, from 2 to 25 months of age. Serum histamine was elevated 1 and 8 hr after challenge. Anti-rice IgE antibodies were demonstrated by radioallergosorbent and Prausnitz-Kustner testing, but not to soy. Rice fluorescent antibody spot tests for anti-rice antibody were positive for IgE, but negative for IgG, IgA and IgM. Precipitation and passive haemagglutination antibody studies were negative for rice and soy. Early in the course serum complement (C) was depressed, increasing to normal only after soy and rice were eliminated. Incubation of patient's serum with rice extract resulted in depressions of haemolytic CH_{50} , C4 and C3 (23, 39 and 28%, respectively). Incubation with soy extract resulted in depression of CH_{50} and C3 only (22 and 27%). Sera from two non-allergic controls produced comparable results. C activation may have occurred *in vivo* following absorption of insufficiently digested foods across an immature gastrointestinal tract. Lethargy and unresponsiveness also occurred early in the course, following ingestion of rice as well as soy.

Introduction

Although rice is usually considered a relatively non-allergenic food (May, 1974), there have been reports of rice intolerance in children (Johnstone, 1959; Ikola, 1963). We studied an infant with gastrointestinal and central nervous system disturbances after rice ingestion who produced IgE antibody to rice. Early in his course, serum complement levels were depressed. *In vitro* studies demonstrated that rice extracts could activate complement directly, in the absence of complement-fixing antibodies. Direct activation of complement by foods may explain the complement depression seen in this infant and perhaps in other infants with food allergies.

Case report

The patient was a normal full-term male infant who was breast-fed during his first

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10 days. A milk-base formula (Similac®) was given for two feedings, but discontinued because of an episode of vomiting. A soy-base formula (Isomil®) was then given and tolerated. At 2 months of age, he developed vomiting and diarrhoea several hours after his first feeding of rice-barley cereal. This cereal was continued with persisting vomiting and diarrhoea and he was hospitalized for dehydration. He improved with intravenous fluids and was asymptomatic when discharged on Isomil®.

At 4 months of age, rice-barley cereal was given again, followed by vomiting, diarrhoea and unresponsiveness. Upon referral to the University of Arizona Health Sciences Center, cereal containing barley was tolerated. Challenge with 20 g of rice baby cereal was followed in 30 min by diffuse flushing and lethargy. At 90 min he was irritable and pale. At 2 and 3 hr repeated, forceful vomiting and explosive, watery diarrhoea began. The stools did not contain reducing substances. At 4 and 6 hr, there were 15 min episodes of lethargy, during which he responded only to deep pain. 10 hr after rice ingestion there was evidence only of mild dehydration. His blood pressure, serum electrolytes, glucose and calcium remained normal throughout. Two subsequent rice challenges reproduced these changes, except the unresponsiveness appeared earlier, beginning 20 min after rice ingestion. An EEG taken during an unresponsive period demonstrated right-sided slowing, but no evidence of paroxysmal discharge. Subsequently, a sleep-tracing was normal. He was discharged on apple sauce, green beans and Isomil®, since these were tolerated.

At 5 months of age, brief periods of extreme lethargy or unresponsiveness began to occur daily. No new foods had been added since discharge. He was readmitted and physical examination, routine laboratory studies, EEG, computerized axial tomography of the head and skull films were normal. Phenobarbital (6 mg/kg per 24 hr) did not alter the clinical pattern and was discontinued, but the spells ceased 24 hr after a protein hydrolysate formula (Pregestimil®) was substituted for Isomil®. 1 week after Pregestimil® was started, a challenge with a soy-based formula was followed within 30 min by extreme lethargy. Challenges with rice at 13, 15 and 25 months of age were followed by flushing, vomiting and diarrhoea without lethargy and unresponsiveness. After 14 months of age, foods containing soy were well-tolerated. Except for rice, all other foods have been introduced without adverse reaction. His growth and development have been normal.

Total serum IgE levels were less than 4 iu/ml at 5 and 8 months of age, and 12 iu/ml at 10 months. IgG levels were low at 6 and 7 months (103 and 140 mg/100 ml, respectively), but normal at 8 and 9 months (390 and 430 mg/100 ml, respectively). Serum IgA levels were normal.

Materials and methods

Complement studies

Functional haemolytic assays of whole complement (CH₅₀), and the fourth (C4) and third (C3) components of complement were performed as described by Rapp & Borsos (1970), Gaither, Alling & Frank (1974) and Pinckard *et al.* (1975), respectively. Immunochemical assays of C3 and properdin factor B were done by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) using antisera obtained from Atlantic Antibodies, Westbrook, Maine. Peripheral blood was collected in EDTA and examined for conversion products of C3 and factor B by crossed antigen electrophoresis (Weeke, 1973) in 1% agarose with 0.01 M EDTA.

The studies on the effect of rice or soy on serum complement (C), *in vitro*, were performed as follows. Food extracts (1:3, w/v), were made in buffered saline with 0.4% phenol using a Polytron 20 (Brinkman Instrument Co., Westburg, New York) as described elsewhere (Pinnas *et al.*, 1977). Protein concentration in the extracts was measured by the method of Lowry *et al.* (1951). Sera from the patient and two control subjects were used. The control subjects were not clinically intolerant of rice and had negative intradermal skin tests with rice extracts. Aliquots of the sera were incubated with equal volumes of extracts of rice (1 mg protein per ml) or soy (60 mg protein per ml), or with diluent alone, for 15 min at 37°C. Functional haemolytic assays of CH₅₀, C4 and C3 were performed on these mixtures. Conversion of factor B to GGG and C3 to B1A was estimated in the same sera.

Serum histamine assay

Serum samples were de-proteinized by the addition of 8% HClO₄, using the technique of Lichtenstein & Osler (1964), as modified by Tannenbaum, Ruddy & Schur (1975). Histamine was determined spectrofluorometrically as described elsewhere (Lichtenstein & Osler, 1964; Hakanson, Ronnberg & Sjolund, 1972).

Antibody studies

Studies for anti-rice and anti-soy antibodies were performed with patient's serum obtained before the rice challenge at 13 months of age. Prausnitz-Kustner (P-K) (Booth, 1972), immunodiffusion (Ouchterlony, 1958) and passive haemagglutination with tanned red cells (Herbert, 1967) were done by standard techniques. Food extracts (1:3, w/v) were as described above.

Indirect immunofluorescence for IgG, IgM, IgA and IgE anti-rice antibodies were performed by the following method. Tanned sheep red cells or tanned red cells coated with rice extract (Herbert, 1967) were cytocentrifuged onto glass slides and then acetone-fixed for 15 min. The patient's serum was layered over the fixed cells for 30 min in a moist chamber and then washed twice for 5 min each time with buffered 0.15 M NaCl (pH 7.2, 0.01 M phosphate). The cells were then incubated with fluoresceinated goat antisera to IgG, IgM, IgA and IgE (Meloy Laboratories, Inc., Springfield, Virginia) for 30 min before washing with buffered saline and examination by fluorescent microscopy, as described elsewhere (Pinnas, Northway & Tan, 1973).

Radioallergosorbent (RAST) tests to rice and soy were kindly performed by Dr Douglas Heiner (University of California at Los Angeles) on serum samples taken at 5 and 9 months of age and by Dr Donald R. Hoffman (Creighton University, Omaha, Nebraska) on a sample taken at 13 months of age, using methods previously described (Hoffman, 1975).

Results

Complement studies

CH₅₀, C4, C3 and factor B levels were measured in sera collected from the patient before and after challenge with rice, while he was taking only soy formula (up to 6 months) and during asymptomatic periods after soy and rice had been eliminated (age 7 to 12 months).

The CH₅₀ levels are shown in Fig. 1 and compared to the rice and soy intake. The

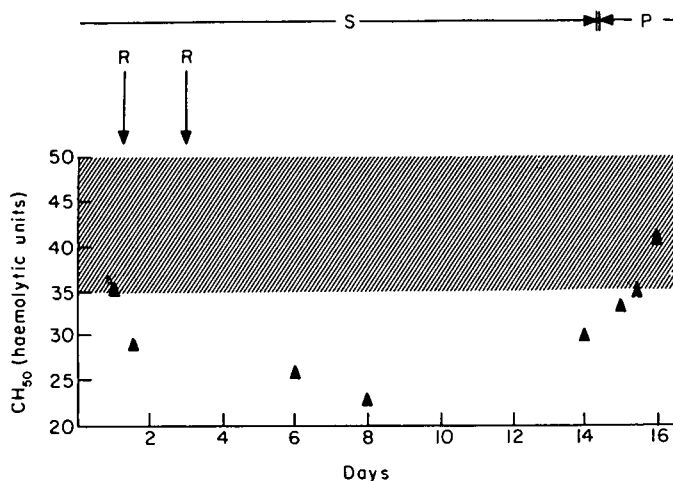


Fig. 1. Whole complement (CH_{50}) levels during hospital admission at 5 months of age. R, Rice challenge, S, soy formula, P, protein hydrolysate formula; shaded area, normal CH_{50} values.

CH_{50} decreased from a borderline normal value to a clearly abnormal value following rice challenge, and did not return to normal until soy was eliminated. During the period when the CH_{50} levels were depressed, C3, C4 and factor B levels were also

Table 1. Serum complement levels*

Serum complement	$n^{\dagger}$	During soy and rice	n	After soy eliminated	P value	Normal range
C3 (mg/dl)	8	90 ± 9.7 (74–104)	6	138 ± 7.8 (124–146)	< 0.001	100–180
C4 (haemolytic units/ml) ($\times 10^{-3}$)	4	200 ± 45 (140–250)	6	520 ± 128 (390–730)	< 0.001	250–600
Factor B (mg/dl)	3	11.7 ± 4.3 (10–14)	6	19.7 ± 3.2 (14–23)	< 0.025	12–30
CH_{50} (haemolytic units)	5	28.8 ± 4.6 (23–35)	6	40.3 ± 5.0 (35–48)	< 0.01	35–50

* Mean $\pm$ 1 s.d. (ranges in parentheses).

† Number of samples.

significantly depressed (see Table 1). No conversion products of C3 or factor B were found in the peripheral blood at 5 months of age, when the CH_{50} was depressed. All levels rose to normal values after soy and rice were eliminated and remained normal over the next 5 months. Separate rice and soy challenges at 13 months of age were not followed by depression of any serum complement components.

The effect of rice or soy extracts on haemolytic C levels, *in vitro*, was similar in the patient's serum and the two control sera. The results of a typical experiment are shown in Table 2. CH_{50} , C4 and C3 levels were all depressed after incubation of the sera with rice extract, when compared to the levels in the sera-diluent mixtures, while

Table 2. Effect of addition of rice or soy upon serum complement, *in vitro*

Serum complement	Solution	Normal serum		Patient serum	
		Units or titre ($\times 10^{-3}$)*	Decrease (%)	Units or titre ($\times 10^{-3}$)*	Decrease (%)
CH ₅₀	Diluent	40.0	—	35.7	—
	Rice	25.3	37	27.4	23
	Soy	31.7	21	27.8	22
C4	Diluent	228	—	187	—
	Rice	125	45	115	39
	Soy	207	9	174	7
C3	Diluent	18.0	—	8.2	—
	Rice	8.8	51	5.9	28
	Soy	10.6	41	6.0	27

* CH₅₀ is in units, C3 and C4 in titre ($\times 10^{-3}$).

only the CH₅₀ and C3 levels were appreciably depressed after incubation with soy extract. Electrophoretic conversion of factor B and C3 occurred consistently. Changes in haemolytic C4 and C3 levels and electrophoretic conversion of factor B and C3 were not observed when the extracts were incubated with serum containing 0.01 M EDTA.

Serum histamine

Serum histamine levels were measured on sera obtained before and after the rice challenge at 13 months of age. The results are shown in Table 3.

Table 3. Serum histamine levels following rice challenge

Time	Histamine (ng/ml)
Pre-challenge	10
1 hr	23
8 hr	17
Normal range	2-10

Antibody studies

P-K testing was performed using the mother as the recipient. Undiluted patient's sera, both fresh and heated (1 hr at 56°C), were used. P-K sites were challenged with rice extract at 4 and 48 hr after injection of the sera. At 4 hr, a reaction was noted at the fresh serum site starting 45 min after the challenge. This reaction, which consisted of itching, redness and induration, reached maximum intensity at 2 hr (25 mm diameter induration, 90 mm diameter erythema) and diminished over the next 10-12 hr. At 48 hr, a similar reaction started 60 min after challenge and was maximal at 90 min (10 mm diameter induration, 15 mm diameter erythema).

Challenges of heated serum P-K sites and non-P-K sites were negative at both 4 and 48 hr. Challenges of fresh serum P-K sites with extracts of soy, oat, wheat and beef were negative.

Rice-coated, tanned red cells which had been reacted with patient's serum and fluoresceinated goat anti-human IgE showed fluorescent staining, but similar preparations in which fluoresceinated goat antisera to human IgG, IgA and IgM were used were negative. There was no fluorescence after reaction of uncoated tanned cells with patient's serum and the fluoresceinated antisera.

Table 4. RAST with rice antigens

Subject	Counts/min		
	5 months*	9 months*	13 months†
Patient	320	370	171
Standard adult control	250‡	250‡	112§
Non-allergic infants¶	n.d.	n.d.	104
			(84-120)**

* RAST performed by Dr Heiner.

† RAST performed by Dr Hoffman.

‡ Fifteen unscreened adults, pooled.

§ Single non-allergic adult.

¶ Seven infants, age 2-14 months.

** Range.

The results of the RAST to rice are shown in Table 4. There was no specific binding to soy, corn, wheat or oat.

Precipitating antibodies could not be detected by immunodiffusion to rice, wheat, corn, soy or oat extracts. Haemagglutinating antibodies to rice or soy also were undetectable.

Discussion

Evidence that this infant is allergic to rice is based upon several observations: (a) repeated challenges with rice over a 23 month period were followed by flushing, vomiting and diarrhoea, (b) serum histamine more than doubled following rice ingestion, rising to a level above the normal range, and (c) anti-rice IgE antibody was detected in serum from the patient by several methods. The IgE antibody to rice was present in low titre, although his total serum IgE remained normal. Studies for antibody to rice other than IgE were negative.

IgE mediation of the reaction after the first exposure to rice at 2 months of age seems likely, but cannot be confirmed. The time of sensitization to rice is unknown, but could have been intrauterine or during breast-feeding. Similac® and Isomil® were the only foods given prior to 2 months of age, and both contain corn oil, but no other grain products. Although the patient had symptoms following ingestion of soy during the first 7 months, the negative P-K and RAST tests and the absence of symptoms with subsequent challenges of soy suggest that the allergy was only to rice.

The delayed onset of the P-K reaction is unusual for an IgE-mediated reaction. Elimination of the reaction by heating and the persistence of the antibody 48 hr after injection of the serum strongly suggest that the reaction was mediated by IgE antibody. Immune adsorption with anti-IgG or anti-IgE prior to the injection of the serum aliquots was not done in order to avoid injection of heterologous serum. The P-K titre was low enough so that monkey skin could not be used as recipient. The small amount of antibody in the patient's serum is probably responsible for the delayed onset of the P-K reaction. Another possibility is that rice may have been modified by local tissue enzymes uncovering the reactive antigenic sites, as has been suggested for certain milk antigens (Spies *et al.*, 1970).

Other features of food intolerance in this infant were unresponsiveness and depressions in serum C, which occurred after ingestion of rice or soy. These findings were both noted early in the course but did not occur after 6 months of age. However, at 13 and 25 months of age, flushing, vomiting and diarrhoea were still observed after rice ingestion. Depression of serum C3 (Matthews & Soothill, 1970) and deposition of C3 in jejunal biopsies (Shiner *et al.*, 1975; Shiner, Ballard & Smith, 1975) have been reported to follow milk challenge in infants with milk allergy. In this infant, depression of C4 and factor B, in addition to the depression of C3 and CH₅₀, indicate that both the classical and alternative C pathways may be involved. The rapid fall of CH₅₀ after the challenge with rice and the rapid increase after soy elimination suggest that C activation had occurred. Failure to detect split products of C3 and factor B in peripheral blood suggest that the activation occurred only in the gastrointestinal tract. Although C-fixing antibodies could account for the depression of serum C, C-fixing antibodies could not be detected in this patient's serum. One may speculate that absorption of undigested substances from rice or soy across an immature GI tract may have directly activated C. *In vitro* studies on the effect of rice or soy on serum C support this suggestion.

In summary, a male infant with rice intolerance had depressed levels of serum C with CNS reactions during his first 6 months of life. Serum anti-rice IgE antibodies were detected. Reports of rice allergy are rare. This study documents that allergy to rice, although rare, does occur and may be associated with gastrointestinal and CNS reactions.

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