

Table 1. Enterovirus-Specific IgG in IVIG Samples

IVIG brand (lot No.)	Mean (SD) enterovirus IgG, ELISA U/mL
Carimmune (1)	193.3 (8.5) ^a
Carimmune (2)	144.7 (6.8)
Gammagard	233.6 (21.1)
Gammunex	245.0 (23.2)
Octagam (1)	189.9 (16.3)
Octagam (2)	221.2 (12.5)

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IVIG, intravenous immunoglobulin.

^a $P = .005$ when compared with Carimmune lot 2.

The kit includes a standard 96-well plate precoated with heat denatured coxsackievirus type B5 and echovirus type 6 virions, which have been shown to cross-react with enterovirus antibodies. Each sample was measured in quadruplicate along with controls. ELISA results were compared between lots using 1-way analysis of variance. Post hoc pairwise comparisons were made using the Tukey test. The protocol was approved by the Department of Clinical Investigation.

Enterovirus IgG was detected in all samples (Table 1). Significant statistical differences were observed between the 2 Carim-

mune lots but not those of Octagam. Each IVIG sample demonstrated statistically significant differences in enterovirus-specific IgG levels when compared with samples from other manufacturers.

Few studies have evaluated enterovirus-specific IgG in IVIG products. Galama et al⁶ assessed enterovirus-specific IgG titers via neutralization assays in 2 IVIG preparations from the Netherlands. They found up to 8-fold differences in titers between the 2 preparations. Dagan et al⁷ evaluated 3 IVIG products via plaque reduction neutralization assays, which revealed no substantial differences among the 3 preparations tested. These results have limited applicability to the IVIG products available in the United States today.

Abzug et al⁸ performed a randomized trial using IVIG (2 lots of Gammagard) in 9 neonates and supportive therapy only in 7 neonates with enteroviral disease and found that IVIG products containing a neutralizing titer of 1:800 or more for the patient's own viral isolate provided a more rapid reduction in viremia and viruria compared with patients who did not receive IVIG or received an IVIG product with enterovirus titers less than 1:800 for the patient's isolate. Faster normalization of clinical findings was demonstrated in the IVIG group. This suggests some benefit in using IVIG preparations with higher titers for the specific enterovirus in infected neonates.

This study has several limitations. A limited number of IVIG samples were evaluated because of supply and cost considerations. Neutralization assay titers and seasonal variation among lots and manufacturers were not assessed. These results cannot be used to directly identify clinically protective levels for any given lot or manufacturer.

The primary objective of this study was achieved in that enterovirus-specific antibodies were present in all brands of IVIG tested. This should provide some reassurance that commercially available IVIG in the United States contains at least some enterovirus-specific antibodies. In the event of enteroviral disease or high-risk exposures, lot-specific measurement of enteroviral-

specific IgG should be considered given the variability identified in this study. We hope that this study will generate interest in disclosing the quantities of enteroviral and other crucial antibodies in each lot of IVIG.

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GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE AS A MAJOR ALLERGEN IN RAMBUTAN-INDUCED ANAPHYLAXIS

Rambutan (*Nephelium lappaceum*) is a widely consumed tropical fruit in Thailand and is exported to many countries worldwide. Currently, only 1 case report of rambutan allergy has been reported, but the allergen has never been identified.¹ This work reports 3 new cases and identification and characterization of a rambutan allergen.

Three adult patients, ages 35, 49, and 53 years, had a history of multiple episodes of anaphylactic reactions within 30 minutes after ingestion of half to 2 rambutans. All patients had cutaneous (eg, angioedema, flushing, and urticaria) and respiratory (eg, rhinitis, chest tightness, stridor, and wheezing) symptoms. One patient also had cardiovascular (eg, syncope) and gastrointestinal (eg, nausea and vomiting) symptoms. Patients 1 and 3 had a history of allergic rhinitis and asthma, respectively. No patient had a history of latex allergy. There was no family history of food allergy. A positive prick-to-prick test result to fresh pulp rambutan was demonstrated in all patients, with mean wheal diameters of 16, 3, and 6 mm, but not in 2 healthy controls. Histamine phosphate (10 mg/mL) and glycinated saline were used as positive and negative controls for skin

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testing, respectively. Skin prick tests using commercial extracts (Center Laboratory, Port Washington, New York) of aeroallergens and foods were performed. Patient 1 had positive reactions to mites, grass, acacia, and careless weeds. Patient 2 had positive reactions to mites, cat, and cockroaches. Patient 3 had positive reactions to mites, grass, shrimp, and crab. Because all of these patients had a history of anaphylaxis to rambutan, a double-blind, placebo-controlled food challenge was not performed for ethical reasons.

Rambutan proteins were extracted from the pulp of ripe rambutans and separated from the peel and seed. Pulp was lyophilized and stirred in extract solution (50mM sodium phosphate, 0.02mM dithiothreitol, and 1mM phenylmethyl sulfonyl fluoride) at 4°C overnight. The extract was centrifuged at 17,210g at 4°C for 30 minutes. The supernatant was filtered and stored at -30°C until used. Total proteins were determined by a bicinchoninic acid protein assay. Rambutan proteins in total extract were resolved in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel under a reducing condition (Figure 1A, lane 1). IgE immunoblot analysis showed all 3 patients had serum IgE bound to approximately 40 kDa of protein, suggesting a major allergen (Figure 1A, lanes 2-4). The spot of approximately 40 kDa of protein was excised from 10% SDS-PAGE gel under a reducing condition (Figure 1A, lane 1) and was electroeluted. Results of IgE immunoblot (Figure 1A, lane 5) and inhibition enzyme-linked immunosorbent assay (Figure 1B) confirmed IgE reactivity of the eluted approximately 40-kDa allergen. The eluted approximately 40-kDa allergen inhibited binding of serum IgE to coated rambutan extract with an

inhibitory concentration that results in a 50% reduction in IgE binding of approximately 13 µg/mL (Figure 1B). Further Mascot search of mass fingerprint of the trypsin-digested, eluted approximately 40-kDa allergen showed 3 matched peptides: AASFNI-IPSTGAAG, VPTVDVSVVDLTVR, and IVSWYDNEWGYSSR of the glycoprotein glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

This is the first study, to our knowledge, to identify GAPDH as the allergen in rambutan. GAPDH of kiwifruit (Act d 3)² and wheat³ was identified as a food allergen, whereas GAPDH of the indoor mold *Aspergillus versicolor* (Asp v 1-3) was a spore allergen.⁴ Although it was suggested that the complex glycans of GAPDH may be cross-reactive carbohydrate determinants for fruits and pollens,² it appears that the cross-reactive carbohydrate determinants are clinically insignificant.⁵ None of the 3 patients had symptoms to wheat or kiwifruit. They also had negative skin test results to wheat and *Aspergillus*. The skin test to kiwifruit was not performed because it was not a common fruit in Thailand.

It was found that, in kiwifruit allergic individuals, high levels of IgE against Act d 1 and 3 (GAPDH) were associated with severe clinical symptoms.² Because all 3 rambutan patients had multiple episodes of anaphylactic reactions after rambutan ingestion, it is possible that these patients might have high levels of IgE against GAPDH.

Fruits in the Sapindaceae family, including rambutan, lychee, longan, and ackee, are reported to cause allergic reactions.⁶⁻⁸ Thus, verifying cross-reactive allergens would be needed. It has been reported that a 16-kDa profilin and a 70-kDa protein are 2 lychee cross-reactive allergens.⁶ Our immunoblotting results revealed that 2 patients had IgE bound to a 70-kDa protein but not to proteins with molecular weights less than 28 kDa (Figure 1A). Because 1 patient also had a history of lychee allergy, further experiments are needed to determine whether GAPDH and the 70-kDa protein are cross-reactive allergens among fruits in the Sapindaceae family.

In conclusion, this is the first study, to our knowledge, that identifies GAPDH as a major allergen in rambutan. Further experiments are needed to examine allergenic determinants of rambutan GAPDH.

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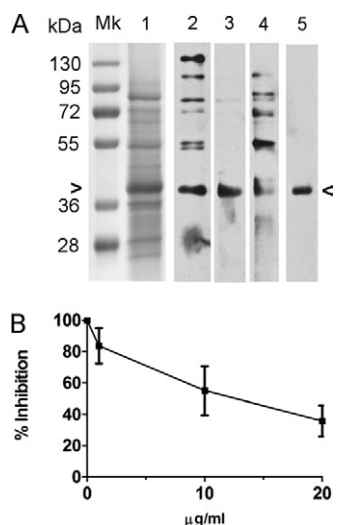


Figure 1. A, Lane 1: sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of total rambutan protein content stained with Coomassie G250. Lanes 2 through 4: immunoblotting of IgE bound proteins in rambutan extract using individual sera from patients 1 through 3. Lane 5: immunoblotting of the IgE-bound, electroeluted approximately 40-kDa allergen. The row marked with > and < indicates the IgE-bound approximately 40-kDa protein. Mk indicates molecular mass markers. B, Inhibition enzyme-linked immunosorbent assay revealed that the electroeluted approximately 40-kDa allergen inhibited IgE binding to coated total rambutan proteins with an inhibitory concentration that results in a 50% reduction in IgE binding of approximately 13 µg/mL.

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ADALIMUMAB DESENSITIZATION AFTER ANAPHYLACTIC REACTION

Adalimumab (Humira) is a recombinant human monoclonal antibody that inhibits tumor necrosis factor α , a substance that plays an important role in the genesis of inflammation.¹ It is used for the treatment of rheumatoid arthritis, ankylosis spondylitis, Crohn disease, psoriatic arthritis, and moderate-severe chronic plaque psoriasis.^{2,3}

The most commonly reported adverse effects are headache, rash, and nausea. In 20% of patients edema, itching, and erythema appear at the injection site.

In addition, hypersensitivity reactions have been reported. According to clinical studies, hypersensitivity reactions to adalimumab have been reported in approximately 1% of treated patients at the recommended therapeutic dose of 40 mg every 2 weeks. There have been rare postmarketing reports of anaphylaxis and/or angioedema.⁴

We report the case of a 50-year-old woman with rheumatoid arthritis. She was initially treated with steroids and nonsteroidal anti-inflammatory drugs, with no improvement in symptoms. Then she began treatment with leflunomide, with the appearance of hives in the late phase. The same symptoms appeared after administration of etanercept. The treatment was discontinued and then replaced with adalimumab.

Initially, the drug was well tolerated, but after 20 minutes from the 10th injection on, erythematous rash and urticaria appeared, which were successfully treated with intramuscular corticosteroids and antihistamines. Within a few minutes of the next dose of adalimumab, the patient experienced the same symptoms, with asthma, facial angioedema, generalized itching, and severe hypotension. She required treatment with intramuscular corticosteroids and antihistamines. Adalimumab therapy was discontinued, and the arthritis worsened.

The patient was referred by rheumatologists to our outpatient department 2 weeks after the reaction to adalimumab. Reports from the emergency department did not indicate the use of adrenaline.

Table 1. Protocol for Rapid Desensitization to Adalimumab

Dose	Concentration	Time	Volume, mL	Administered dose, mg	Cumulative dose, mg
1	1/1	0	0.1	5	5
2	1/1	30'	0.2	10	15
3	1/1	30'	0.2	10	25
4	1/1	30'	0.3	15	40

Tryptase during the reaction were not administered. The baseline tryptase test result was negative.

Skin prick tests with adalimumab were performed at least 3 weeks after the initial adverse reaction to minimize false-negative results. Monoclonal antibody was obtained as a full-strength solution or reconstituted per the manufacturer's instructions and was diluted further in normal saline for intradermal testing. Histamine prick (10 mg/mL) was used a positive control. For epicutaneous skin testing, a drop of adalimumab (50 mg/mL) was applied and pricked to the volar surface of the forearm. It elicited an unclear response because of dermographism. Then we proceeded with intradermal injections of 0.03 mL of a 1:100 and 1:10 dilution of the adalimumab strength solution, which produced negative results.

Given the clinical success of adalimumab, the patient, in agreement with the rheumatologist, agreed to be re-treated with this drug. Because of the severity of the initial reaction, despite the negative skin test results, we decided to proceed with the readministration using a desensitization protocol, as reported by Brennan et al in 2009.⁵

After informed consent was obtained from the patient, desensitization was performed in the outpatient department. The patient was monitored for vital signs, and the intensive unit care was alerted in case of anaphylactic reactions. She was premedicated with cetirizine, 10 mg, and montelukast, 10 mg, 30 minutes before the protocol.⁶ The protocol began with an initial subcutaneous dose of 5 mg (1/1), which was gradually increased until a cumulative dose of 40 mg was reached (Table 1). Dose escalation occurred every 30 minutes. The complete process took 2 hours, and the patient did not develop local or systemic reactions.

In the second week, the patient received 20 mg plus 20 mg after 30 minutes, with premedication, and then she received the full dose at once in the third and fourth weeks. She experienced a significant clinical improvement during the following weeks. The patient continued the treatment every 2 weeks, as suggested by her rheumatologist. Currently, she has been receiving therapy for 2 years without any adverse effects.

Our desensitization protocol provided for the attainment of the therapeutic dose in only 2 hours, with premedication (antihistamines and antileukotrienes) for the first 4 sessions.

In patients with moderate to severe reactions, suggestive of IgE-mediated reactions, even if doubt or negative skin test results occur, induction or tolerance is a recommended option. In the literature, there are some reports of desensitization protocols with other biological drugs but only 1 report with adalimumab for generalized urticaria and rhinitis. In this case, the desensitization was performed with a standard protocol, with achievement of the maximum dose in 6 hours.⁷

We report the first case, to our knowledge, of anaphylactic shock with adalimumab, with inconclusive skin test results. The

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