CASE REPORTS

Identification of a 27 kDa Protein in Patients With Anaphylactic Reactions to Mango

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■ Abstract

Mango fruit has become increasingly popular in recent years. We report on 2 patients who developed anaphylactic reactions after the ingestion of fresh mango. Allergy to mango was confirmed by a positive skin prick test result and positive cellular allergen stimulation test results. Neither of the patients had detectable mango-specific immunoglobulin (Ig) E levels. Results were validated by sodium dodecyl sulfate polyacrylamide gel electrophoresis immunoblotting and analyzed using Quantiscan. We identified 2 major allergens with a molecular weight of 27 kDa in both patients, in addition to a 15 kDa allergen in 1 patient and a 32 kDa allergen in the other. Currently available IgE systems seem to be lacking these mango allergens and as such are probably unsuitable for diagnosing type 1 sensitization to mango. Skin prick testing with fresh mango fruit therefore seems to be a much more reliable test method for clinical practice.

Key words: Mango. Anaphylaxis. Cross-reactivity. Pollen-associated food allergy.

■ Resumen

El mango se ha convertido en una fruta de creciente popularidad. Presentamos 2 pacientes que desarrollaron reacciones anafilácticas tras la ingesta de mango fresco. La alergia a mango se confirmó por la positividad en el test cutáneo positivo y el test de estimulación alergénica celular. Ninguno de los pacientes presentaban niveles detectables de inmunoglobulina E (IgE) específica frente a mango. Los resultados fueron validados mediante electroforesis en (SDS-PAGE) gel de poliacrilamida dodecil-sulfato sódico e inmunoblotting (inmunodetección) y analizándolos mediante Quantiscan. Identificamos 2 alérgenos principales con un peso molecular de 27kDa en los dos pacientes, además de un alérgeno de 15kDa en uno de los pacientes y un alérgeno de 32kDa en el otro paciente.
Los sistemas de IgE disponibles actualmente parece que carecen de estos alérgenos del mango y por esto son probablemente poco apropiados para el diagnóstico de la sensibilización de tipo I frente al mango. El test cutáneo con mango en fresco parece, por tanto, el método diagnóstico más fiable en la práctica clínica.

Palabras clave: Mango. Anafilaxia. Reactividad cruzada. Alergia alimentaria asociada a polen.

Introduction

Mango belongs to the Anacardiaceae family (*Sumac* species), which also includes cashews and pistachios. The fruit (*Mangifera indica L.*) is used in various dishes and has become increasingly popular in Europe in recent years. Rubin and Shapiro [1] were the first to report an anaphylactic reaction following the ingestion of mango; since then, the fruit has been reported to cause both type 1 allergy (anaphylaxis or oral allergy syndrome) and type 4 allergy (contact dermatitis) [2]. Mango allergens have also been shown to cross-react with mugwort/celery, latex (via class I chitinases), papaya, tomato, and banana [3,4].

Mango allergy appears to be more common than is

generally believed; in a French study conducted in a group of 580 patients with allergic reactions to different foods, 6% of patients reacted to mango, which in many cases was a "hidden" ingredient in commercial food products [5]. Here we report on 2 German patients who developed type 1 allergy to mango.

Case Description

Patient 1

A 46-year-old female with atopy developed sneezing attacks, rhinorrhea, dyspnea, dysphagia, and anxiety several minutes after drinking a mango fruit shake containing fresh ginger. A similar reaction had occurred in the past after the patient had eaten a fresh mango. The patient developed severe anaphylactic reactions involving rhinorrhea, dyspnea, and cardiopulmonary symptoms on 2 other occasions: the first time after drinking a multivitamin fruit juice containing occult mango juice and the second time after eating a pistachio bar. The patient also reported intermittent allergic rhinitis in September and October.

Patient 2

A 24-year-old male developed acute generalized urticaria and deep-tissue swelling of the hands and the face after consuming fresh mango and an alcoholic liquor containing different herbal essences. Two years earlier, he had developed similar symptoms after eating a fruit salad containing fresh mango. The patient had received specific immunotherapy several years earlier to treat known mugwort sensitization.

Neither of the patients complained of any symptoms after contact with latex, celery, carrots, or other spices.
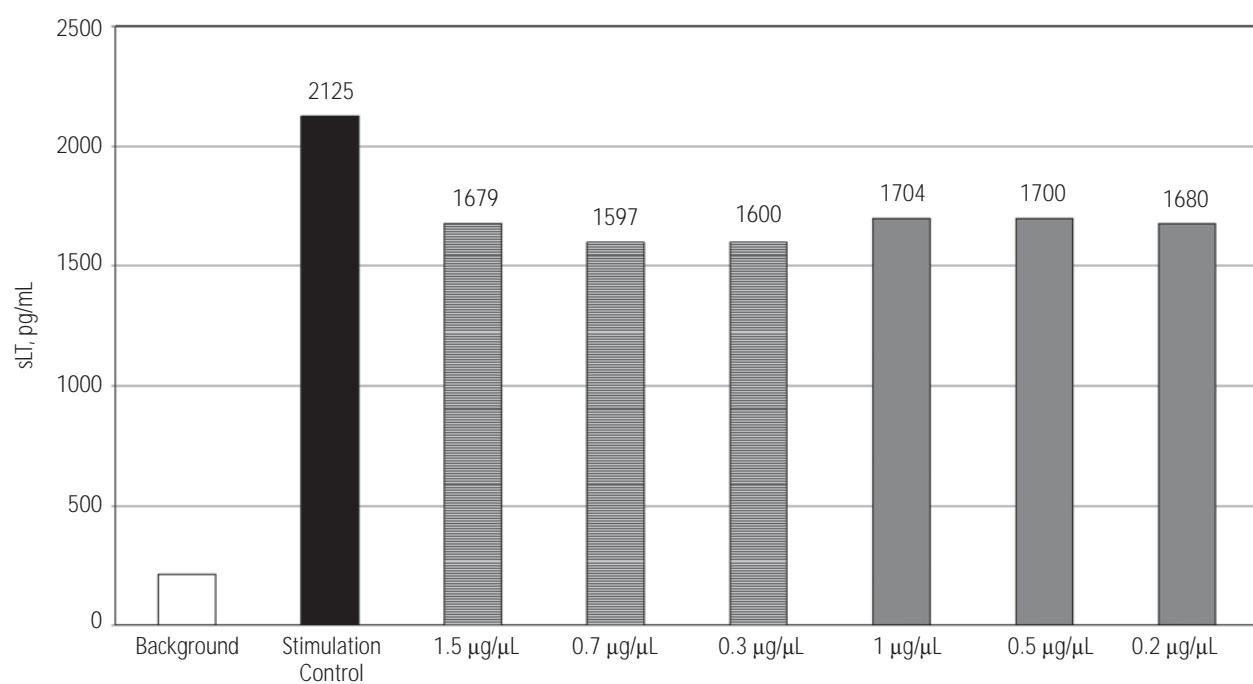
Material and Methods

Both patients underwent skin prick testing (SPT) with a standard series of inhalant allergens and fresh preparations of mango, ginger, pistachio, soy milk, celery, and carrot. Patient 2 was also tested with the herbal liquor he had drunk. A skin prick wheal was considered positive when its diameter was 3 mm larger than that produced by a positive histamine control and a negative sodium chloride control.

Table. Skin Prick Test Results for Patients 1 and 2 With Corresponding Specific Immunoglobulin (Ig) E Antibody Levels

Allergens	Wheal/Flare Size, mm (Patient 1)	Specific IgE, kU_A/L (Patient 1)	Wheal/Flare Size, mm (Patient 2)	Specific IgE, kU_A/L (Patient 2)
Mugwort (w6)	4/9	3.61	4/15	15.0
Hazel (t4)	4/6	1.07	ND	0.56
Birch (t3)	2/0	rBetv2/rBetv4 = 1.48 rBetv1 < 0.35	4/6	rBetv2/rBetv4 < 0.35 rBetv1 = 0.49
Alder (t2)	3/4	1.06	ND	0.95
Rye (g12)	3/5	0.74	5/12	1.84
Grass (g6)	3/4	0.48	3/5	1.04
Latex (k82)	–	< 0.35	–	< 0.35
Ginger (f270)	4/10	< 0.35	–	< 0.35
Mango (f91)	5/20	< 0.35	6/18	< 0.35
Pistachio (f203)	5/13	< 0.35	5/16	< 0.35
Soy milk (f14)	3/5	< 0.35	ND	< 0.35
Celery (f85)	–	< 0.35	5/11	< 0.35
Carrot (f31)	–	0.51	ND	< 0.35
Alcoholic liquor containing *Meum athamanticum*	ND	ND	3/5	ND
Alternaria alternate (m6)	ND	< 0.35	5/15	11.7
Ragweed (w1)	ND	5.23	5/18	8.70
Histamine	5/10	–	5/16	–
Total IgE	32.4 kU/L		122 KU/L	

Abbreviation: ND, not done.

A

Cellular Antigen Stimulation Test (CAST) With Mango and Mugwort Extract (Patient 2)
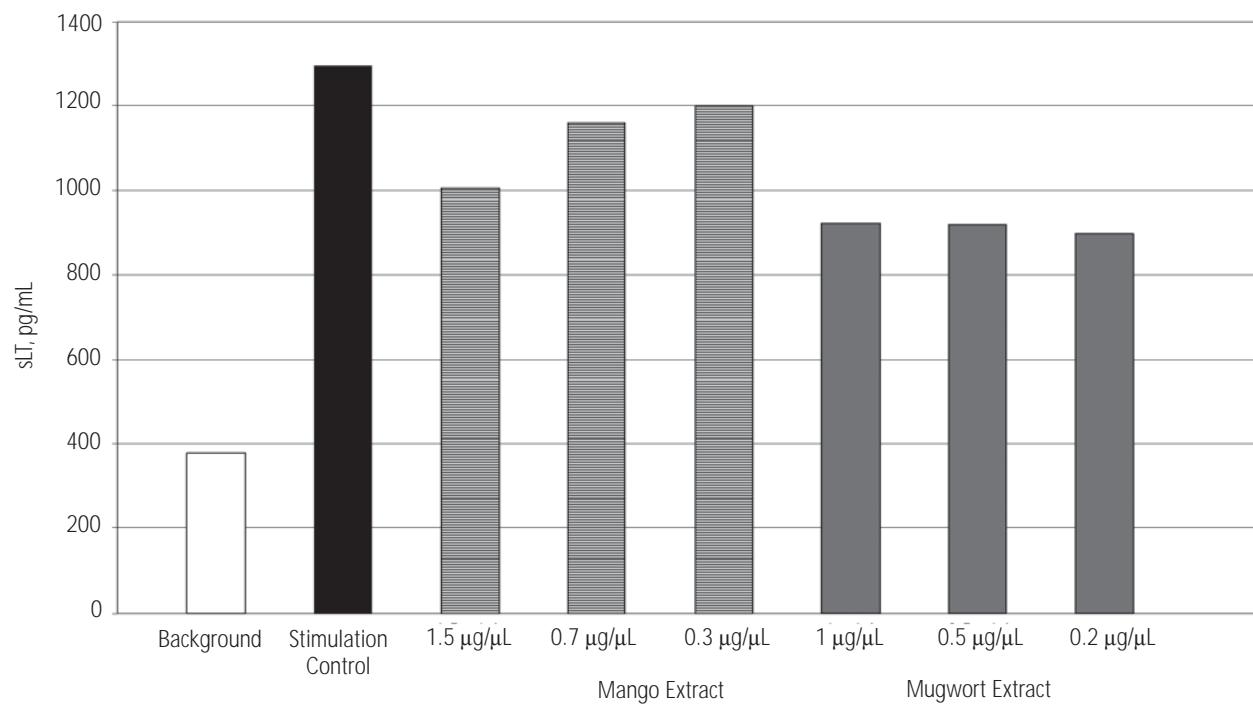
B

Figure 1. A, CAST results with mango extract for patient 1. B, Corresponding CAST results for patient 2. Sulfidoleukotriene (sLT) levels were significantly elevated in both cases.
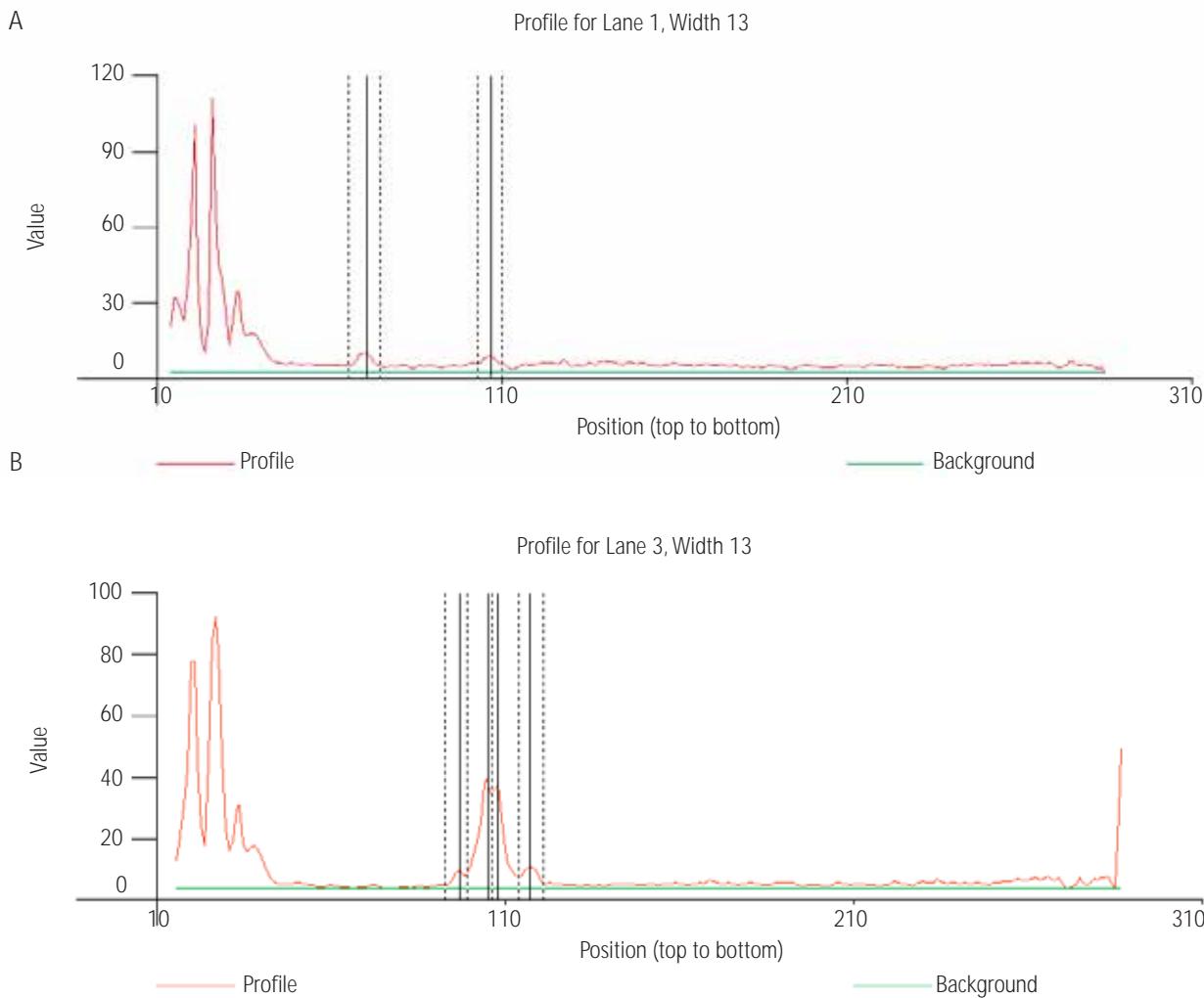
A

Profile for Lane 1, Width 13

B

Profile for Lane 3, Width 13

Figure 2. A, immunoblot analysis for patient 1 showing 2 major allegens (27 kDa and 15 kDa). B, Corresponding analysis for patient 2 (major allergens of 27 kDa and 32 kDa).

Total immunoglobulin (Ig) E, specific IgE, and serum tryptase levels were validated using the CAP-fluorescence enzyme immunoassay (FEIA) system (Phadia, Freiburg, Germany) and AlaSTAT kits (DPC Biermann, Bad Nauheim, Germany). Radioallergosorbent (RAST) inhibition was performed using an inhibition buffer solution and patient serum preincubated with mango extracts. Mango-specific IgE-antibodies were then measured using the CAP-FEIA system. Analyses were performed with absolute values and the percentage of inhibition observed for the sample was correlated with control values [6,7].

Mango extracts were prepared according to the procedure described in [8] and tested and verified by homologous inhibition. The cellular antigen stimulation test (CAST) was performed using previously prepared mango extract, and EDTA-treated blood was obtained by dextran sedimentation. After incubation at room temperature for 90 minutes, the upper phase was centrifuged, the supernatant removed, and the cell pellet resuspended in 2 mL of stimulation buffer with interleukin 3. The buffer solution was used as a negative control and high-affinity monoclonal antibodies against the IgE Fc-receptor as a positive control [9]. Fifty μL of the allergen solutions were then added. The CAST was performed using the CAST-ELISA kit (Bühlmann Laboratories AG, Basel, Switzerland) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli [10]. Protein bands were visualized by staining the gel with Coomassie brilliant blue. After SDS-PAGE immunoblotting, the proteins were transferred using a well described procedure involving diffusion to a nitrocellulose sheet and subsequently incubated with patient serum [11]. Bound-specific IgE antibodies from patients can be detected with the addition of alkaline phosphatase-conjugated substrate. To characterize the molecular weight of the single marked
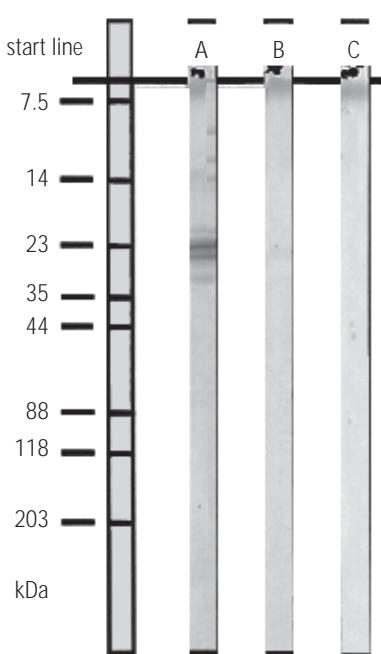
Figure 3. Heterologous and homologous inhibition of mango in corresponding immunoblot analysis. A indicates control; B, mango; C, mugwort.

lanes, a reference molecular weight marker was used for comparison purposes. Inhibitions were also conducted with mango and mugwort in the immunoblots containing the 27 kDa allergen.

Discussion

More complete allergy evaluations and tests are becoming increasingly important with the growing popularity of exotic fruits in Europe and the increasing prevalence of mango allergy. In 2 patients who developed a typical anaphylactic reaction after ingesting mango, we were unable to detect mango-specific IgE antibodies (both < 0.35 kU$_A$/L) using commercially available CAP systems, even though both patients had clearly positive SPT results (5/20 mm and 6/18 mm, respectively). The 2 patients also had other allergic reactions, the most serious of which was to mugwort pollen. The results of our allergy evaluation, which included SPT and allergen-specific IgE detection are given in the Table. Tryptase levels were within the normal range for both patients.

SPT results were confirmed using the CAST method, which showed that sulfidoleukotriene values were significantly higher (by 3- to 7-fold) than unstimulated control values (see Figure 1). We used homologous RAST-inhibition to validate the mango extract used.

Finally, SDS-page identified a 27 kDa protein in both patients. On analyzing the subsequent blots with Quantiscan

(Biosoft, Cambridge, UK), we identified 2 major allergens with a molecular weight of about 27 kDa in both patients, as well as an allergen of 15 kDa in patient 1, and one of 32 kDa in patient 2 (see Figure 2). Moreover, the 27 kDa peak was inhibited by mugwort (heterologous inhibition) in the immunoblot (Figure 3). We thus confirmed our clinical hypothesis of type 1 sensitization to mango in both patients.

In 46 out of 52 patients with IgE-mediated sensitization to mango fruit, Paschke et al [12] identified 2 major heat-stable allergens of approximately 40 kDa (*Mangifera indica* 1) and 30 kDa (*Mangifera indica* 2). We found major allergens of 27 kDa in both of our patients as well as a 15 kDa allergen in patient 1 and a 32 kDa allergen in patient 2.

Dube et al [13] confirmed that the allergenicity of mango puree and nectar persists even after heating, mechanical tissue disintegration, and enzymatic decomposition. As occurred in our case, specific IgE tests may be negative in patients with clear clinical evidence of mango allergy because the corresponding allergens are unstable or simply do not exist in currently available commercial tests [14-16]. Henzgen et al [14] reported on 6 patients with definite clinical symptoms and positive SPT results, but only 1 of these had mango-specific IgE antibodies. Immunoblot inhibition identified several possible mango-specific allergens: a 15 kDa protein (thought to be profilin) in 1 patient and a 45 kDa and a 94 kDa protein in 2 patients. One of our patients also had a major 15 kDa protein in the immunoblot. The IgE detection systems currently available on the market appear to be lacking these specific mango allergens and as such do not seem to be appropriate for diagnosing type 1 sensitization to mango.

Anaphylactic reactions to mango may be due not only to specific epitopes but also to cross-reacting epitopes in pollen-allergic patients. Both of our patients had anaphylactic symptoms attributed to type 1 sensitization to mango, but they also had polyvalent sensitization to pollen (mostly mugwort).

Several antigens are responsible for cross-reactions between mango and other plants and fruit, mostly involving allergens related to Bet v1, Bet v6, and Art v1 [17]. In our case, patient 1 showed slightly elevated levels of carrot-specific IgE antibodies (0.51 kU$_A$/L) (CAP 1) but did not develop any clinical signs of anaphylaxis after eating uncooked carrots. We interpreted this as sensitization without clinical relevance.

Pistachio belongs to the same family as mango and cross-reactivity between both has been reported [3,15]. Interestingly, both of our patients had positive SPT results for pistachio (patient 1, 5/13 mm and patient 2, 5/16 mm), and patient 1 also reported clinical anaphylactic symptoms after ingestion of pistachio.

Surprisingly, 1 of the patients also had a positive SPT result for ginger (4/10 mm) and it is not clear whether this sensitization may have contributed to the clinical reactions observed. Ginger belongs to a different family (Zingiberaceae) to mango. IgE-dependent anaphylactic-type reactions caused by fresh ginger have not been described in the literature to date. There are, however, reports of IgE-mediated asthma [18] and eczematous reactions in chronic hand eczema in cooks [19].

In view of the likelihood that standard commercially available IgE detection systems are lacking certain mango

allergens, we believe that SPT is the best method for confirming mango allergy.

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