Development and Evaluation of a Rapid Assay for the Diagnosis of Immunoglobulin E-Mediated Type I Allergies

R Lucassen,[1] M Fooke,[1] J Kleine-Tebbe,[2] M Mahler[1]

[1]Dr Fooke Laboratorien GmbH, Neuss, Germany
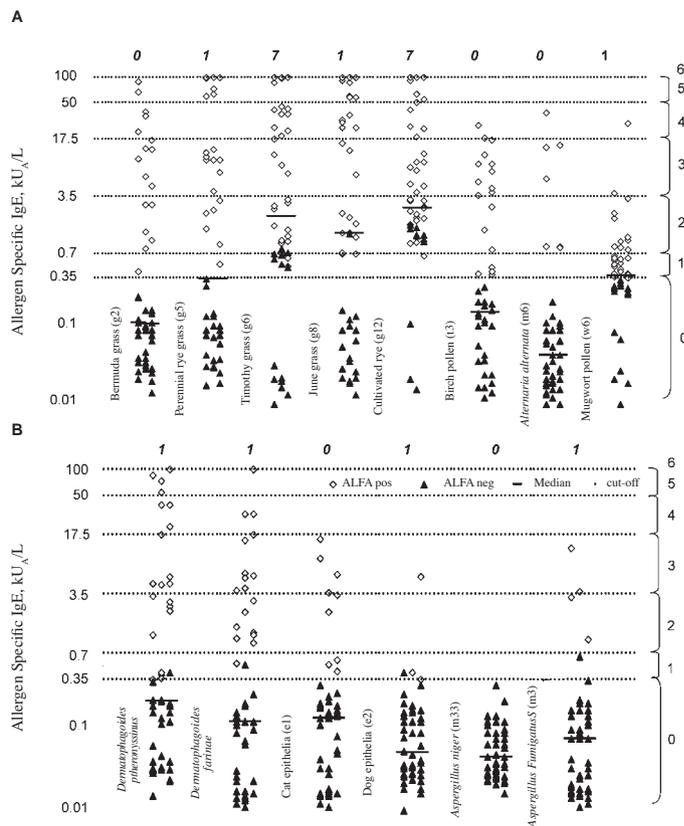[2]Allergy and Asthma Center, Westend, Berlin, Germany

Figure. Serologic characterization of the sample cohort. Allergen-specific immunoglobulin (Ig) E profiles of samples (n=50) for (A) ALFA Seasonal Screen and (B) ALFA Perennial Screen were generated by ALLERG-O-LIQ system. Bold figures indicate the number of ALLERG-O-LIQ-positive but ALFA-negative samples.

A characteristic feature of type I allergies is the presence of allergen-specific immunoglobulin E (sIgE) [1,2]. Thus, the detection of sIgE, in addition to obtaining a clinical history and performing a skin prick test (SPT), is important for allergy workup. Historically, sIgE to various allergens was analyzed by radioallergosorbent test using allergen-coupled cellulose paper discs. Later on, the enzyme allergosorbent test and the reversed allergosorbent test were used for the detection of sIgE [3]. In recent years, rapid assays for the detection of sIgE as point-of-care diagnostics have been developed using various strategies [4]. The objective of this study was the technical evaluation of 2 rapid assays (ALFA Seasonal Screen [ALFA S] and ALFA Perennial Screen [ALFA P]) for the detection of sIgE to the most common inhalant allergens.

Serum samples (n=50) were tested by ALFA and sIgE was analyzed for all single allergens utilized in ALFA allergen screens by ALLERG-O-LIQ (Dr. Fooke Laboratorien GmbH, Neuss, Germany), a reverse-type, quantitative immunoassay (WHO 75/502 calibrated). The maximum kU_A/L value (ALLERG-O-LIQ) of each sample was used as a reference value. For comparison of ALLERG-O-LIQ and ALFA, samples were defined as ALLERG-O-LIQ positive when the sIgE result was ≥ 0.35 kU_A/L. Appropriate statistics were used.

sIgE profiles of specimens were generated by ALLERG-O-LIQ. Twenty-six (52.0%) samples were positive for sIgE to at least 1 perennial and 45 (90.0%) to at least 1 seasonal allergen. Twenty-four (92.3%) of the 26 perennial-positive samples were also positive by ALFA P and 38 (84.4%) of the 45 seasonal-positive samples were also positive by ALFA S. When a cutoff value of 1.6 kU_A/L was used, the sensitivity increased to 100% for both ALFA tests. None of the ALLERG-O-LIQ-negative samples tested positive using the respective ALFA assay (100% specificity). The kappa statistic was 0.92 (P<.0001) for ALFA P and 0.52 (P=.0003) for ALFA S compared to the ALLERG-O-LIQ. A serologic characterization of the sample cohort is shown in the Figure.

In recent years, sIgE profile and screening tests have been developed using different protocols. In 2004, comparison of ALLERG-O-LIQ and the ImmunoCAP system showed good agreement for inhalant allergens and moderate agreement for food allergens [5]. ImmunoCAP Rapid, which is also based on lateral-flow technology, has been shown to yield results that are concordant with clinical diagnosis [4]. The major difference between ALFA and ImmunoCAP Rapid tests is that ALFA utilizes liquid allergen mixtures while ImmunoCAP Rapid employs single allergens immobilized on membranes.

Recent studies have provided evidence that the number of positive sIgE results and the total amount of sIgE correlate with disease severity and the number of clinical symptoms [6,7]. Therefore, screening tests using allergen mixtures may reflect the "allergic risk" of a patient. Notably, in this study, 9 samples with low sIgE titer (≤ 1.6 kU_A/L) were ALFA negative and ALLERG-O-LIQ positive. This is consistent with reports in the literature indicating that low sIgE titers do not necessarily imply clinical relevance [8].

Although SPT represents a reliable method for allergy diagnosis, it has some drawbacks, including serious side

effects [9,10]. Based on these observations, rapid allergy tests may represent a promising alternative to SPT, although further studies will be necessary to confirm this. ALFA is available as a doctor's office test for pediatricians and primary care physicians and as an over-the-counter test for home users, providing a new tool for type I allergy diagnosis. This might lead to a reduction in the number of patients undergoing laborious testing and thus reduce the overall costs for the health care system.

References

1. Ishizaka K, Ishizaka T. Identification of γE-Antibodies as a Carrier of Reaginic Activity. J Immunol. 1967;99:1187-98.

2. Johansson SG. Raised levels of a new immunoglobulin class (IgND) in asthma. Lancet. 1967;4:951-3.

3. Hamilton RG, Franklin Adkinson N Jr. In vitro assays for the diagnosis of IgE-mediated disorders. J Allergy Clin Immunol. 2004;114:213-25.

4. Eigenmann P, Kuenzli M, D'Apuzzo V, Kehrt R, Joerg W, Reinhardt M, Carlsson L, Borres M, Lauener R. The ImmunoCAP RapidWheeze/Rhinitis Child test is useful in the initial allergy diagnosis of children with respiratory symptoms. Allergy. 2007;62:128-9.

5. Kleine-Tebbe J, Breuer K, Lepp U, Vieths S, Worm M, Kunkel G, Wahn U, Lau S. Spezifische IgE-Werte gegen Aero- und Nahrungsmittelallergene im Vergleich: Dr. Fooke ALLERG-O-LIQ und Pharmacia CAP-System. Allergologie. 2004:27:129-35.

6. Nopp A, Johansson SG, Lundberg M, Oman H. Simultaneous exposure of several allergens has an additive effect on multisensitized basophils. Allergy. 2006;61:1366-8.

7. Wickman M, Lilja G, Soderstrom L, van Hage-Hamsten M, Ahlstedt S. Quantitative analysis of IgE antibodies to food and inhalant allergens in 4-year-old children reflects their likelihood of allergic disease. Allergy. 2005;60:650-7.

8. Dolen WK. IgE antibody in the serum--detection and Flanders diagnostic significance. Allergy. 2003;58:717-23.

9. Liccardi G, D'Amato G, Canonica GW, Salzillo A, Piccolo A, Passalacqua G.J Systemic reactions from skin testing. J Investig Allergol Clin Immunol. 2006;16:75-8.

10. Liccardi G, Salzillo A, Spadaro G, Senna G, Canonica WG, D'Amato G, Pasalacqua G. Anaphylaxis caused by skin prick testing with aeroallergens: Case report and evaluation of the risk in Italian allergy services. J Allergy Clin Immunol. 2003;111:1410-2.

Michael Mahler, PhD
Dr. Fooke Laboratorien GmbH
Maintraße 85
41469 Neuss, Germany
E-mail: mmahler@fooke-labs.de

Poppy Seed Anaphylaxis

J Panasoff
Allergy Department, Lin Medical Center, Clalit Health Services, Haifa, Israel

Poppy seeds, which are obtained from the poppy plant *Papaver somniferum*, are sometimes used in bakeries, especially for cakes, in Europe and the Middle East. Yet, despite widespread use, they rarely cause allergic reactions.

Recently, a 17-year-old boy presented with a history of a generalized reaction soon after eating a poppy seed cake. The reaction consisted of acute abdominal pain quickly followed by diffuse urticaria and low blood pressure (70/45). He improved after being promptly treated with adrenaline and corticosteroids, but about an hour afterwards he complained again of severe abdominal pains that subsided after receiving a new dose of adrenaline. A couple of weeks later, he felt a tingling and burning sensation in his mouth after eating cheesecake. He remembered that this cake was close to a poppy seed cake. He was sure the cakes were on different plates and were cut into portions using different knives.

When he was 10 years old' he suffered twice from allergic reactions (urticaria and dyspnea) that apparently were severe enough to require adrenaline. Both reactions occurred after eating at restaurants. According to the family, an allergy workup was negative, but since then he has noticed that eating nuts causes a mild local reaction in his mouth.

Skin tests with commercial extracts (ALK-Abelló, Port Washington, New York, USA) showed moderate reactions to Brazil nuts and pistachios. A skin prick test with an inhouse extract, prepared using a small quantity of raw poppy seed diluted in phenol saline, was highly positive and also produced a transient mild reaction consisting of headaches and abdominal pain. Parallel tests with this extract were performed on his mother and staff members and the results were negative. Specific immunoglobulin (Ig) E testing (Immulite 2000, 3gAllergy™, Siemens Medical Solutions Diagnostics, New Jersey, USA) was highly positive and confirmed his hypersensitivity to poppy seeds (28.4 kU/L). It also showed that he was sensitized to hazelnuts (35.10 kU/L), Brazil nuts (10 kU/L), sesame (15.20 kU/L), and tomato (9.23 kU/L). As he reported eating sesame products and tomato without problems, these reactions were deemed clinically nonrelevant. At our request, the patient spoke with the bakeries that prepared the cakes involved in these reactions and they confirmed that the cakes contained no nuts. The patient was advised to carry Epipen (Dey LP, Napa, California, USA) and to avoid poppy seeds, hazelnuts, pistachios, and Brazil nuts.

The simultaneous presence of IgE sensitization to poppy seeds with other kinds of food has been described elsewhere: these include buckwheat [1], sesame, and hazelnut [2,3].

Although cross-sensitization is suspected, it has been shown [4] that poppy seeds, hazelnuts, rye grain, sesame, and kiwi have cross-reacting and unique components that make it possible to have simultaneous and clinically relevant allergy to poppy seeds and other food products. It is also noteworthy that a reaction to poppy seed can be caused not only orally but also by inhalation [1]. This is a possible explanation for the milder second reaction reported by this patient.

This case confirms that poppy seed allergy, although rare, is usually severe. As there are few commercially available tests, skin testing with an inhouse preparation is a feasible option to confirm diagnosis.

References

1. Oppel T, Thomas P, Wollenberg A. Cross-sensitization between poppy seed and buckwheat in a food-allergic patient with poppy seed anaphylaxis. Int Arch Allergy Immunol. 2006;140(2):170-3.
2. Keskin O, Sekerel BE. Poppy seed allergy: a case report and review of the literature. Allergy Asthma Proc. 2006 Jul-Aug;27(4):396-8.
3. M, Kägi M, Wüthrich B. Poppy seed anaphylaxis. Schweiz Med Wochenschr. 1995 Jul 29;125(30):1434-7.
4. Vocks E, Borga A, Szliska C, Seifert HU, Seifert B, Burow G, Borelli S. Common allergenic structures in hazelnut, rye grain, sesame seeds, kiwi, and poppy seeds. Allergy. 1993 Apr;48(3):168-72.

Josef Panasoff
Allergy Department
Lin Medical Center
Clalit Health Services
35 Rostchild Avenue
Haifa, Israel
E-mail: josefpa@clalit.org.il

Failure of Omalizumab Treatment After Recurrent Systemic Reactions to Bee-Venom Immunotherapy

V Soriano Gomis, P Gonzalez Delgado, E Niveiro Hernandez.
Allergy Section, General University Hospital of Alicante, Alicante, Spain

Venom immunotherapy is used in children and adults with a documented sensitization to a particular insect and who have had severe systemic reactions [1]. The great majority of patients with systemic reactions are allergic to bee venom [2]. During specific immunotherapy with hymenoptera venoms, systemic reactions are not uncommon, especially during the initial phase. For this reason the therapeutic schedule may have to be modified or treatments may even have to be discontinued. Omalizumab, a recombinant humanized monoclonal antibody against immunoglobulin (Ig) E offers a new therapeutic approach for the treatment of allergic diseases. Currently, this drug has been approved for the treatment of severe allergic asthma. However, other allergic disorders may be amenable to treatment with omalizumab because of its ability to inhibit effector functions of IgE [3]. We explored a role for this agent in combination with bee-venom immunotherapy in an attempt to reach a maintenance dose in a patient with recurrent systemic reactions to immunotherapy.

The patient was a 27-year-old man who had experienced his first allergic reaction following a bee sting in adolescence. The reaction consisted of urticaria, dyspnea and hypotension. An allergy study showed a positive intradermal skin test to bee venom at 0.0001 µg/mL (10^{-7} g/L) and negative reaction to a vespid venom; the concentration of serum specific IgE (ImmunoCAP, Phadia, Uppsala, Sweden) against bee venom was 12.90 kU/L. Total serum IgE was 161 IU/mL. We prescribed bee-venom immunotherapy with a conventional schedule. During the incremental phase he suffered a systemic allergic reaction (urticaria and hypotension) at a cumulative dose of 10 µg/mL of venom. Therefore we recommended measures to avoid new bites, prescribed adrenaline self injection, and scheduled follow-up. Two years later we repeated bee-venom immunotherapy, again with a conventional schedule, and the patient suffered an identical systemic reaction at the same dose as before. Three years later we again tried to reach a maintenance dose with bee-venom immunotherapy, again with a conventional schedule but without melittin (Aquagen, ALK-Abelló, Madrid, Spain). However a systemic reaction developed again at 10 µg/mL of venom, with urticaria and hypotension.

Considering the high risk to the patient we looked for an alternative treatment. In another allergy study skin reactivity was the same as before, and specific IgE to bee-venom was detected at a concentration of 11.80 kU/L; the tryptase concentration was 2.2 µg/L. Total serum IgE was 168 IU/mL. The patient was prescribed 300 mg of omalizumab once a month for 6 months, in accordance with his weight and level of total serum IgE. The serum specific IgE to bee-venom increased to 95.60 kU/L and total serum IgE reached 795 IU/mL. Two weeks after the last dose of omalizumab we started bee-venom immunotherapy with an ultra-rush schedule and pretreatment with 180 mg of fexofenadine daily for 1 week. However, the patient suffered yet another systemic reaction at a cumulative dose of 10 µg/mL of venom, with erythema and hypotension. Specific immunotherapy could not be increased and was stopped.

Although venom immunotherapy reduces the risk of future anaphylactic reaction, a maintenance dose cannot be reached in some patients who develop a systemic reaction to venom immunotherapy. These patients are usually allergic to bee venom and high sensitivity in the skin test may be an

indicator of possible systemic reaction [2]. Recently the side effects of specific bee-venom immunotherapy were reported to be reduced by anti-IgE [4] and pretreatment with omalizumab in ragweed-allergic patients has been shown to improve the safety profile of specific rush immunotherapy [5].

We have reported a case of systemic allergic reaction with bee-venom immunotherapy in spite of pretreatment with omalizumab and antihistamines. We administered this drug with the aim of decreasing the level of free IgE in serum without regard to allergen specificity for reducing the risk of anaphylactic side effects after immunotherapy injections. Although there is the possibility that the non-tolerance of the extract was due to our use of an ultra-rush schedule, in which the reaction occurred at the same cumulative dose as in the 4 previous systemic reactions, we believe that omalizumab is still far from providing a preventive treatment. In specific situations, the allergic reaction persists.

References

1. Bonifazi F, Jutel M, Bilo BM, Birnbaum J, Muller U. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy. 2005;60:1459–70.
2. Rzany B, Przybilla B, Jarisch R, Aberer W, Dietschi R, Wüthrich B, Bühler B, Frosch P, Rakoski J, Kiehn H, kUhn W, Urbanek R, Ring J. Clinical characteristics of patients with repeated systemic reactions during specific immunotherapy with hymenoptera venoms. Allergy. 1991;46:251-4.
3. Vaishali S, Mankad A, Burks W. Omalizumab, other indications and unanswered questions. Clin Rev Allergy Immunol. 2005;29:17-30.
4. Schulze J, Rose M, Zielen S. Beekeepers anaphylaxis: successful immunotherapy covered by omalizumab. Allergy. 2007;62:963-4.
5. Casale TB, Busse WW, Kline JN, Ballas ZK, Moss MH, Townley RG, Mokhtarani M, Seyfert-Margolis V, Asare A, Bateman K, Deniz Y. Immune Tolerance Network Group. Omalizumab pretreatment decreases acute reactions after rush immunotherapy for ragweed-induced seasonal allergic rhinitis. J Allergy Clin Immunol. 2006;117:134-40.

Victor Soriano Gomis
Sección de Alergia
Hospital General Universitario de Alicante
C/ Maestro Alonso, 109
0310 Alicante, Spain
E-mail: soriano_vic@gva.es; vsoriano@meditex.es

Allergic Contact Dermatitis Due to a Temporary Henna Tattoo, a Hair Dye, and a Marker Pen

MA Gonzalo-Garijo,[1] DA Fernández-Durán,[2] R Pérez-Calderón,[1] J Sánchez -Carvajal[1]
[1]*Department of Allergology, Infanta Cristina University Hospital, Badajoz, Spain*
[2]*Servicio de Dermatología, Hospital La Princesa, Madrid, Spain*

Recently, temporary henna tattoos have become popular in Western populations and there has been a parallel increase in the number of reports of allergic contact dermatitis to such tattoos. Most cases have been related to p-phenylenediamine (PPD) added to the traditional mixtures of henna [1,2].

An 18-year-old woman had a temporary henna tattoo applied to her right arm by a street vendor in Marrakech, Morocco. Four days later, she developed an itchy erythematous papulovesicular reaction at the site of the tattoo. Following treatment with corticosteroid creams, the lesions healed within 2 to 3 weeks, leaving postinflammatory hypopigmentation that followed the original design (Figure). Four months later she applied a hair dye at home, and the next day she developed severe facial edema and a pruriginous papulovesicular eruption at the hairline. She was admitted to hospital and treated with systemic antihistamines and corticosteroids, and gradually improved over the following week. The patient had never used hair or textile dye products and she had never applied henna tattoos previously.

Patch testing was performed 6 weeks later with the GEIDC (Spanish Contact Dermatitis Research Group) standard series, 3 types of commercial henna powder (10% aqueous solutions),

Figure. Postinflammatory hypopigmentation following the original design of a henna tattoo.

and a textile dyes series. Readings at 48 and 96 hours were positive (+++) to PPD, black rubber mix, disperse yellow 3, disperse orange 1 and 3, and disperse red 1 and 17. We confirmed the presence of PPD as an ingredient of the hair dye, but it was not possible to obtain the complete list of ingredients of the paste used for the temporary tattoo. Interestingly, during patch testing the patient also developed a pruritic vesicular eruption where a marker pen had been used (Schneider 232, Schneider Schreibgeräte GmbH, Tennenbronn, Germany). The reaction appeared 48 hours after the application and faded within 4 to 5 days. The manufacturer of the marker pen provided us with the ink components: ethanol, 1-methoxy-2-propanol, Solvent Black 7, Solvent Violet 8, Solvent Orange 3, and phosphoric acid, mono bis (2-ethylhexyl) ester. Ethanol, 1-methoxy-2-propanol, and phosphoric acid, mono bis-(2-ethylhexyl)-ester had been tolerated by the patient. Patch tests with ink from the Schneider marker and 2 black markers from different manufacturers, Solvent Black 7, Solvent Violet 8, and Solvent Orange 3 (all of them at a concentration of 1% in petrolatum) were positive (++) at 48 and 96 hours to the Schneider marker and Solvent Orange 3. The same marker pen had been used in 40 patients with no reactions. The manufacturer of the marker emphasized that it is not intended for use on the skin.

Despite its widespread use, reports of contact dermatitis to pure henna are very rare. It can therefore be assumed that henna is a very weak skin sensitizer. However, the addition of PPD to the traditional mixture has been the main cause of the numerous reported reactions to temporary tattoos. PPD is a potent sensitizer, and patients who are allergic to this substance may develop a sensitization to related compounds with similar structures, such as azo dyes, parabens, para-aminobenzoic acid, and para compounds [1,2], as was the case in our patient. This could explain the reaction to ink from a marker pen that included Solvent Orange 3, an azo-derivative dye. Although there is a high incidence of multiple sensitizations to related dyes in patients sensitized to PPD from temporary tattoos [2], allergic contact dermatitis to skin markers has rarely been reported. Among the allergens identified, there are some dyes such as Solvent Blue 36 [3] and Solvent Yellow 146 [4]. To our knowledge, this is the first report of a case of allergic contact dermatitis to Solvent Orange 3 in marker pen occurring as a consequence of sensitization to PPD contained in a temporary henna tattoo.

References

1. Wolf R, Wolf D, Matz H, Orion E. Cutaneous reactions to temporary tattoos. Dermatol Online J [serial on the Internet]. 2003 [cited 2007 Dec 14]; 9:3. Available from: http://dermatology.cdlib.org/91/original/henna/wolf.html.

2. Matulich J, Sullivan J. A temporary henna tattoo causing hair and clothing dye allergy. Contact Dermatitis. 2005;53:33-6.

3. Miller MM, Goldberg HS, Wilkerson WG. Allergic contact dermatitis to 1,4-bis(isopropylamino)anthraquinone caused by a felt-tip marker pen. Arch Dermatol. 1978;114:1793-4.

4. Komericki P, Kern T, Aberer W, Kränke B. Contact dermatitis from Solvent Yellow 146 in a permanent marker. Contact Dermatitis. 2001;44: 256.

M. Angeles Gonzalo-Garijo
Hospital Universitario Infanta Cristina
Servicio de Alergología
Carretera de Portugal s/n
06080 Badajoz, Spain
E-mail: magonzalog@telefonica.net

Milkflower cotoneaster (*Cotoneaster lacteus*): A New Lipid Transfer Protein Source From the Rosaceae Family

JA Torres,[1] C Escudero,[1] MD Ibáñez,[1] A Ledesma,[2] M Lombardero[2]
[1]*Allergy Department, Hospital Infantil Universitario Niño Jesús, Madrid, Spain*
[2]*ALK-Abelló, SA, Research and Development Department, Madrid, Spain*

Lipid transfer lipoproteins (LTPs) have been identified as major allergens from fruits in the Rosaceae family in Mediterranean countries [1]. They are the main allergens identified in Rosaceae fruit allergy without associated pollinosis [2].

A 9-year-old girl developed hives and itching on her face and neck. A few minutes before appearance of the symptoms, she had been touching some red berries from a bush in a park. She did not eat the fruit and had no other symptoms. The reaction disappeared later after intake of an oral antihistamine. She had not eaten any food or taken any medication before the episode; nor had she been engaged in intense exercise. Four months earlier, she had experienced 2 episodes of facial urticaria while peeling and eating a peach. She reported tolerance of other fruits from the Rosaceae family (cherry, plum, apple, apricot, and strawberry).

Leaves and red berry samples from the suspicious plant were examined to determine that the plant they came from was milkflower cotoneaster (*Cotoneaster lacteus*), which belongs to the Rosaceae family.

Skin prick tests (prick-to-prick method) were negative to *C lacteus* leaves and to a kit of common inhalants including pollens (ALK-Abelló, SA, Madrid, Spain), but positive to peach, apricot, cherry, and the *C lacteus* red berry (peel of the berry: 7 mm; pulp: 3 mm,). A skin prick test with purified palm profilin (ALK-Abelló) was negative. A rubbing test with the *C lacteus* berry was also positive. Four nonpollinic, peach-allergic patients used as controls were tested by prick-to-prick with the *C lacteus* berry; the results were positive in 3 of them. The total serum immunoglobulin (Ig) E concentration was

17 kU/L. Specific-IgE determination (ImmunoCAP, Phadia, Uppsala, Sweden) for peach was positive (5.78 kU/L).

The *C lacteus* berry was extracted for 90 minutes at 4ºC with 1.8% sodium chloride; the mixture was centrifuged and the supernatant filtered and stored at –20ºC. The *C lacteus* red berry protein extract was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in non-reducing conditions. IgE-immunodetection (Figure, part A) with the patient's serum (lane 3) showed a binding band at about 14 kDa corresponding to the *C lacteus* berry extract, which was also detected in 2 of the control sera (lanes 4 and 7). There was a second IgE-binding band of about 10 kDa observed in control patient 4 (lane 7). The molecular weight of these allergens suggested that they could belong to the LTP family [1]. The presence of a protein homologous to Pru p 3 (peach LTP) in the red berry of *C lacteus* was confirmed by recognition of a band of about 14 kDa when SDS-PAGE was performed in this extract using a Pru p 3-specific polyclonal rabbit antiserum (Figure, part B). IgE-specific levels in the patient's sera against Pru p 3 and Mal d 4 (apple profilin) were measured using an ADVIA Centaur (Bayer, Munich, Germany) immunoassay platform [3]. All the patient sera showed specific-IgE levels to Pru p 3 but were negative to profilin.

This is the first report of contact urticaria due to *C lacteus*. Results of the in vitro assays suggest that LTP from the *C lacteus* fruit was responsible for the cutaneous symptoms of this patient. Moreover, 2 of the 4 nonpollinic, peach-allergic control sera recognized the same IgE-binding band in the *C lacteus* red berry extract. LTPs are 9-15 kDa polypeptides, mainly located on fruit skins and have a defensive function in plants. They are thermostable and resistant to pepsine digestion, making them potent food allergens [4,5]. A history of allergic reactions after handling *C lacteus* berries should be taken seriously and investigation of this allergen should be recommended as part of the diagnostic work-up for nonpollinic, peach-allergic patients.

References

1. Sánchez Monge R, Lombardero M, García Sellés FJ, Barber D, Salcedo G. Lipid transfer proteins are relevant allergens in fruit allergy. J Allergy Clin Immunol. 1999;103:514-9.
2. Fernandez-Rivas M, van Ree R, Cuevas M. Allergy to Rosaceae fruits without related pollinosis. J Allergy Clin Immunol. 1997;100:728-33.
3. Petersen AB, Gudmann P, Milvang-Gronager P, Morkeberg R, Bogestrand S, Linneberg A, Johansen N. Performance evaluation of a specific IgE assay developed for the ADVIA Centaur® immunoassay system. Clin Biochem. 2004;37:882-92.
4. Asero R. Detection and clinical characterization of patients with oral allergy syndrome caused by stable allergens in Rosaceae and nuts. Ann Allergy Asthma Immunol. 1999; 83:377-83.
5. Salcedo G, Sánchez-Monge R, Díaz-Perales A, García-Casado G, Barber D. Plant non-specific lipid transfer proteins as food and pollen allergens. Clin Exp Allergy. 2004;34:1336-41.

J.A. Torres
Sección de Alergología
Hospital Infantil Universitario Niño Jesús
Avenida Menéndez Pelayo, 65
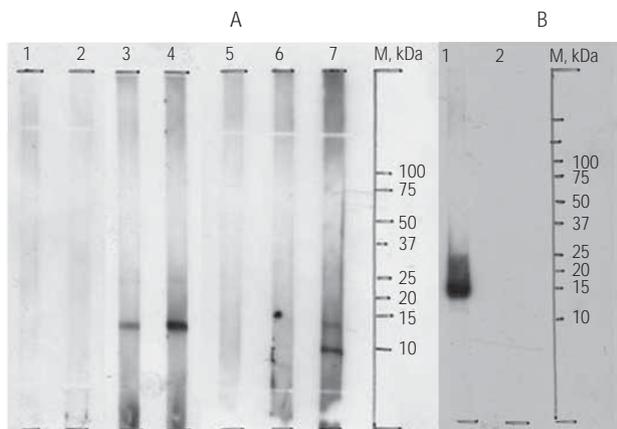28009 Madrid, Spain
E-mail: jath09@yahoo.com

Figure. A: Immunoglobulin E immunodetection of *Cotoneaster lacteus* red berry extract: lane 1, negative control with buffer; lane 2, pool of sera from non-allergic patients; lane 3, study patient; lane 4, control patient 1; lane 5, control patient 2; lane 6, control patient 3; lane 7, control patient 4. B: Immunodetection of *C lacteus* extract with a Pru p 3-specific polyclonal rabbit antiserum (lane 1) or with buffer (lane 2). Molecular weights are indicated in kilodaltons.

Scampi Allergy: From Fancy Name-Giving to Correct Diagnosis

DG Ebo, CH Bridts, MM Hagendorens, LS De Clerck, WJ Stevens
Department of Immunology-Allergology-Rheumatology, University Hospital Antwerp, University of Antwerp, Belgium

Diagnosis of allergy from crustaceans generally relies upon the clinical history and positive specific immunoglobulin (sIg) E and/or skin prick test (SPT). However, taking a history can be hampered by the misuse of popular names. We observed 20 patients with a compelling history of allergy that was initially erroneously attributed to scampi, or Norwegian lobster (*Nephrops norvegicus*). However, a careful reading of the

package leaflets revealed that the reactions must have actually resulted from a giant freshwater prawn (*Macrobrachium rosenbergii*). Furthermore, as is the case for giant freshwater prawn, diagnosis can be hindered by the absence of validated sIgE assays and skin prick test (SPT) extracts.

This study assesses whether the basophil activation test (BAT, for review [1,2]) could confirm allergy caused by giant freshwater prawn, and whether commercial sIgE and SPT for shrimp and recombinant shrimp tropomyosin [3] can act as surrogate markers to diagnose giant freshwater prawn allergy. The results were compared with a newly developed sIgE assay (ImmunoCAP, Phadia, Uppsala, Sweden) for shrimp mixture and giant freshwater prawn.

Most patients had immediate systemic reactions. Twenty-five individuals who were tolerant for giant freshwater prawn and other crustaceans served as controls.

Total and sIgE testing for shrimp (*Pandalus borealis*), shrimp mixture (*P borealis, Penaeus monodon, Metapenaeus joyneri,* and *Metapenaeopsis barbata*), giant freshwater prawn, and recombinant tropomyosin from *Penaeus aztecus* were performed with the ImmunoCAP FEIA technique (Phadia AB, Uppsala, Sweden). sIgE values greater than or equal to 0.35 kUa/L were considered positive.

SPT was performed with shrimp (*Penaeus setiferus*, HAL Laboratories, Haarlem, the Netherlands). A wheal/flare reaction greater than 3/3 mm was considered positive.

Flow cytometric analysis (FACScan, BD, Immunocytometry Systems, San Jose, California, USA) of basophils was performed using Alexa 448-coupled anti-IgE (Sigma-Aldrich, Chemic GmBH, Steinheim, Germany) and double labeling with phycoerythrin-conjugated anti-CD63 (Pharmingen, BD Biosciences, Erembodegem, Belgium) [4]. BATs used a negative control, a positive control (anti-IgE), and a dialyzed extract from giant freshwater prawn, prepared as described by Alenius et al [5].

Receiver operating characteristic (ROC) curve analysis was performed to calculate the thresholds between patients and controls.

The table summarizes the total IgE, sIgE, and SPT results. A positive sIgE result for shrimp, shrimp mixture, giant freshwater prawn, and tropomyosin was observed in 65%, 80%, 65%, and 25% of the patients, respectively. Three controls demonstrated a shrimp sIgE. Respectively, 3 and 2 out of 20 controls demonstrated a positive sIgE result for shrimp mixture and giant freshwater prawn. No sensitization for tropomyosin was demonstrable in controls. Sensitivity of the shrimp SPT was 38%.

Preliminary dose-finding experiments (10 controls, 10 patients) demonstrated dose-dependent CD63 upregulation in basophils relative to spontaneous expression in the patients when activated with giant freshwater prawn, whereas in controls, CD63 expression remained unaltered and reflected spontaneous expression (data not shown). ROC analysis generated a threshold value of 4%, 6%, and 8% for stimulation at 1, 10, and 100 μg/mL, respectively. The corresponding sensitivity and specificity of the BAT were 71% and 100% for 1 μg/mL, 100% and 100% for 10 μg/mL, and 93% and 99% for 100 μg/mL.

Table. Characteristics of Patients and Results of Skin Testing and Immunoglobulin E Assay

Sex	Symptoms	Total IgE	Specific IgE (kUa/L)				SPT	Shrimp Allergy as Reported by Patient	BAT[a]
			Pandalus borealis	Shrimp mix	Tropomyosin	*Macrobrachium rosenbergii*	*Penaeus setiferus*		
M	B	1469	3.45	3.85	0.46	2.7	+	+	66
F	U, A	230	4.46	0.69	<0.35	1.8	-	?	85
M	U, A, B	53	<0.35	0.43	<0.35	<0.35	+	?	14
F	OAS	565	4.54	2.74	1.41	4.07	+	+	70
M	U, A	982	4.14	1.25	<0.35	0.46	+	?	31
M	U, A, B, H	376	4.62	0.79	<0.35	0.43	-	?	46
F	G, A	115	8.99	6.41	<0.35	3.33	-	?	45
M	U	47	<0.35	0.65	<0.35	1.03	NA	?	15
F	U	336	5.22	0.51	<0.35	<0.35	-	+	39
M	U, A, B	319	<0.35	<0.35	<0.35	0.60	-	+	52
F	U, A	303	7.49	6.47	8.14	NA	+	+	64
F	U, A, B, H	104	<0.35	<0.35	<0.35	<0.35	-	+	14
F	U, A, C	22	<0.35	<0.35	<0.35	<0.35	-	+	15
F	OAS	483	0.56	0.49	<0.35	<0.35	+	+	48
M	U, A	2680	30.60	9.79	0.40	3.20	-	?	55
F	U, B	60	3.22	1.47	<0.35	<0.35	-	+	37
F	U, A, B	753	<0.35	12.7	<0.35	NA	-	?	53
M	A	1022	0.38	1.14	<0.35	3.71	-	?	90
M	U, S	112	<0.35	<0.35	<0.35	0.53	NA	+	81
F	U, A	293	2.95	2.60	1.98	NA	+	+	12

Abbreviations: A, angio-edema; B, bronchospasm; C, conjunctivitis; F, female; G, gastro-intestinal symptoms; H, hypotension; Ig, immunoglobulin; M, male; NA, not available; OAS, oral allergy syndrome; S, shock; SPT, skin prick test; U, urticaria. +, positive; -, negative; ?, unknown.
[a] Results expressed as percentage CD63+ basophils.

The table shows the percentages of CD63 in patients for a stimulation concentration at 10 µg/mL. In controls, the maximal CD63 expression was 6%.

In the absence of an sIgE assay and skin test extract for giant freshwater prawn, one might expect sIgE and/or SPT for other crustaceans, particularly shrimp, to be useful surrogate markers. Our data show that this practice is not appropriate. Approximately one-third of our patients had negative sIgE results for shrimp, whereas almost two-thirds demonstrated negative SPTs for shrimp. Alternatively, the newly developed sIgE assay using shrimp mixture attained a sensitivity of 80% and specificity of 85%. All but 5 patients were sIgE-negative for tropomyosin, thus fueling the hypothesis that giant freshwater prawn allergy in our patients probably does not result from sensitization to this muscle protein.

Our data show the BAT to be a reliable instrument for diagnosing allergy caused by giant freshwater prawn, provided the correct allergen is selected.

In conclusion, allergy caused by crustaceans can be severe and an accurate diagnosis is mandatory to avert further reactions. Correct identification of the causal agent is not only hampered by the absence of sensitive and specific skin test extracts and sIgE assays, but also by the misuse of popular names. The BAT and sIgE for shrimp mixture are the most reliable instruments for diagnosing allergy from giant freshwater prawn. In our patient group, allergy from giant freshwater prawn does not seem to result from sensitization to tropomyosin.

Acknowledgments

References

1. Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ. In vitro allergy diagnosis: should we follow the flow? Clin Exp Allergy. 2004;34:332-9.
2. Ebo DG, Sainte-Laudy J, Bridts CH, Mertens CH, Hagendorens MM, Schuerwegh AJ, De Clerck LS, Stevens WJ. Flow-assisted allergy diagnosis: current applications and future perspectives. Allergy. 2006;61:1028-39.
3. DeWitt AM, Mattsson L, Lauer I, Reese G, Lidholm J. Recombinant tropomyosin from Penaeus aztecus (rPen a 1) for measurement of specific immunoglobulin E antibodies relevant in food allergy to crustaceans and other invertebrates. Mol Nutr Food Res. 2004;48:370-9.
4. Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ. Flow cytometric analysis of in vitro activated basophils, specific IgE and skin tests in the diagnosis of pollen-associated food allergy. Cytometry B Clin Cytom. 2005;64:28-33.
5. Alenius H, Kalkkinen N, Lukka M, Reunala T, Turjanmaa K, Makinen-Kiljunen S, Yip E, Palosuo T. Prohevein from the rubber tree (Hevea brasiliensis) is a major latex allergen. Clin Exp Allergy. 1995;25:659-65.

Wim Stevens
Department of Immunology
University of Antwerp
Campus drie Eiken
Universiteitsplein 1
2610 Antwerpen, Belgium
Tel.: ++ 32 (0) 3 8202595
Fax: ++ 32 (0) 3 8202655
E.mail: immuno@ua.ac.be