

Squillace of the Allergic Diseases Research Laboratory, The Mayo Clinic, Rochester, Minnesota, for their work determining the ovalbumin content in the seasonal influenza vaccine lots.

LAURA E. HOWE, MD*
ANNA S. C. CONLON, MS†
MATTHEW J. GREENHAWT, MD, MBA‡
GEORGIANA M. SANDERS, MD, MS‡

*Departments of Pediatrics and Internal Medicine
†School of Public Health
‡Division of Allergy and Clinical Immunology
University of Michigan
Ann Arbor, Michigan
gsanders@umich.edu

1. Sicherer SH, Sampson HA. Food allergy. *J Allergy Clin Immunol*. 2010;125:S116–S125.
2. Kelso JM. Administration of influenza vaccines to patients with egg allergy. *J Allergy Clin Immunol*. 2010;125:800–802.
3. Greenhawt M, Li JT, Bernstein DI, et al. Administering influenza vaccine to egg allergic recipients: a focused practice parameter update. *Ann Allergy Asthma Immunol*. 2011;106:11–16.
4. Chung EY, Huang L, Schneider L. Safety of influenza vaccine administration in egg-allergic patients. *Pediatrics*. 2010;125:e1024–e1030.
5. Gagnon R, Primeau MN, Des Roches A, et al. Safe vaccination of patients with egg allergy with an adjuvanted pandemic H1N1 vaccine. *J Allergy Clin Immunol*. 2010;126:317–323.
6. Greenhawt M, Chernin A, Howe L, Li J, Sanders G. The safety of H1N1 influenza A vaccine in egg allergic children. *Ann Allergy Asthma Immunol*. 2010;105:387–393.
7. Li JT, Rank MA, Squillace DL, Kita H. Ovalbumin content of influenza vaccines. *J Allergy Clin Immunol*. 2010;125:1412–1413.

CONTACT URTICARIA TO RAW ARUGULA AND PARSLEY

A 24-year-old woman with a history of seasonal rhinoconjunctivitis presented with severe angioedema of the lips and tongue, associated with oropharyngeal pruritus and choking sensation, which developed approximately 5 minutes after the ingestion of a few raw arugula leaves. The acute reaction was successfully managed at an emergency unit with intramuscular epinephrine (0.5 mL of a 1:1,000 solution) and intravenous administration of chlorpheniramine. A similar episode occurred 2 months earlier after she ate a pizza with tomato and raw arugula. Moreover, the patient reported previous episodes characterized by lip angioedema after the consumption of foods containing raw parsley, as well as the occurrence of itchy erythematous-edematous lesions of the hands after contact with uncooked leaves of the specified vegetables. On the other hand, the patient did not report any symptoms after the ingestion of cooked parsley or arugula.

Skin prick tests (SPTs) were performed with common airborne allergens and with a large panel of commercial food extracts (Lofarma, Milan, Italy). The SPT result was assessed as positive if the wheal diameter was 3 mm or larger than that of the negative controls. Positive results were observed with *Parietaria*, *Dermatophagoides pteronyssinus*, cat dander, and grass. Furthermore, prick-prick testing was performed with leaves of arugula and parsley and showed strong positive results, eliciting a wheal of 12 mm for the former and 11 mm for the latter. Ten healthy volunteers tested with the same procedure had negative results. Specific IgE for parsley (2.39 kUA/I), *D pteronyssinus*, grass, and *Parietaria* were detected by ImmunoCAP, whereas specific IgE test results for rBet v 1 were negative. IgE antibodies for arugula have not been investigated because the ImmunoCAP test for this vegetable is not available.

Parsley and arugula leaves were extracted as previously described by Bjorksten et al.¹ Protein content was determined according to Bradford² using a commercial BioRad Protein Assay Dye Reagent (BioRad, Milan, Italy) and bovine serum albumin as reference standard. The protein concentration was 1.1 mg/mL and 931 µg/mL for parsley and arugula extracts, respectively.

Parsley and arugula extracts were dialyzed extensively against double distilled water, lyophilized, and then dissolved in prick solution (0.68% sodium chloride, 0.275% sodium bicarbonate, 50% glycerol, and 0.4% phenol) at a concentration of 80 µg/mL. The SPTs performed with both allergen extracts showed a strong positive reaction, with a 12-mm wheal for the parsley extract and a 10-mm wheal for the arugula extract. Extracts at 1:10 dilution showed positive reactions, with a wheal of 8 mm for both vegetables. Ten healthy volunteers tested with the same procedures had negative results.

Parsley and arugula extracts were mixed with lithium dodecyl sulfate sample buffer (Nupage Bis-Tris; Invitrogen, Milan, Italy) and 5% β-mercaptoethanol. The samples were then denatured by heating at 100°C for 10 minutes. Electrophoresis of both extracts (25 µg per lane) was performed in a 10% polyacrylamide precast gel (Nupage Bis-Tris) at 180 mA for 1 hour. The gel was stained with a colloidal blue staining kit (Invitrogen). Immunoblotting showed no IgE reactivity in the patient's serum.

Eruca sativa, commonly known as arugula, rocket, or roquette, is an edible annual plant that belongs to the family Brassicaceae or Cruciferae and is used as a leaf vegetable, either raw or cooked, in some Mediterranean countries, especially Italy. Parsley (*Petroselinum crispum*) is a herbaceous plant widely used in cooking that belongs to the Apiaceae family, which is not botanically related to Brassicaceae.

Allergic reactions to the ingestion of these vegetables seem to be rare. Previous reports have described the occurrence of urticaria-angioedema after eating parsley.³ In 1 case IgE reactivity against a 12-kDa lipid transfer protein was demonstrated by immunoblotting.⁴ Regarding arugula, a case documented the occurrence of IgE-mediated urticaria after eating or skin contact with this vegetable.⁵ Another report described a patient sensitized to *Eruca sativa* who developed intraoral and respiratory symptoms after its ingestion.⁶ In our case, contact urticaria to both vegetables was established based on history, positive SPT results, and positive ImmunoCAP results for the parsley. The patient appeared to tolerate the ingestion of cooked arugula and parsley, as frequently observed in patients with fruit-pollen syndrome.⁷ Immunoblotting assay failed to detect any IgE reactivity against the protein components of each vegetable in the patient's serum. A possible explanation of the difference between ImmunoCAP and immunoblotting could be that the extract sample for immunoblotting was previously heated (5 minutes at 100°C). We cannot exclude that this treatment can alter allergenic epitopes, which consequently makes them no more recognizable by IgE testing of the patient's serum.

Disclosures: Authors have nothing to disclose.

© 2011 American College of Allergy, Asthma & Immunology.

Published by Elsevier Inc. All rights reserved.

doi:10.1016/j.anai.2011.01.029

CATERINA FOTI, MD*
 NICOLETTA CASSANO, MD*
 GIANNI MISTRELLO, MD†
 STEFANO AMATO, MD†
 PAOLO ROMITA, MD*
 GINO ANTONIO VENA, MD*

*Department of Dermatology
 MDIM
 Bari, Italy
 †Lofarma SpA
 Milano, Italy
 c.foti@dermatologia.uniba.it

1. Bjorksten F, Halmepuro L, Hanuksela M, Lahti A. Extraction and properties of apple allergens. *Allergy*. 1980;35:671–677.
2. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72:248–254.
3. Kauppinen K, Kousa M, Reunala T. Aromatic plants: a cause of severe attacks of angio-edema and urticaria. *Contact Dermatitis*. 1980;6:251–254.
4. Cordobés-Durán C, García-Menaya JM, Lombardero M, Ledesma A, Bobadilla P. Detection of a 12-kilodalton lipid transfer protein allergen in parsley. *J Invest Allergol Clin Immunol*. 2007;17:282–283.
5. Pigatto PD, Bigardi A, Fumagalli M, Legori A, Altomare GF. Ig-E-mediated contact and generalized urticaria from *Eruca sativa*. *Contact Dermatitis*. 1991;25:191–192.
6. Liccardi G, Salzillo A, Mistrello G, Roncarolo D, D'Amato M, D'Amato G. Intraoral and respiratory allergy to *Eruca sativa* (Cruciferae family) in a subject with sensitization to pollen allergens. *J Allergy Clin Immunol*. 1998;101:559–560.
7. Asero R, Monsalve R, Barber D. Profilin sensitization detected in the office by skin prick test: a study of prevalence and clinical relevance of profilin as a plant food allergen. *Clin Exp Allergy*. 2008;38:1033–1037.

SEASONAL EOSINOPHILIC BRONCHITIS DUE TO ALLERGY TO CUPRESSUS ARIZONICA POLLEN

Nonasthmatic eosinophilic bronchitis (NAEB) is characterized by chronic cough and sputum eosinophil counts higher than 2.5% of total cells in the absence of reversible airflow limitation and airway hyperresponsiveness.¹ Sporadic cases of work-related NAEB have been reported,² the diagnosis being confirmed when significant increases in eosinophils counts in induced sputum are found after exposure to the offending agent (either in the workplace or after specific inhalation challenge [SIC]) compared with baseline.² However, detailed profiles of sensitizing allergens have not been reported, and the cause usually remains unknown.

Two nonsmoking patients (a 34-year-old woman and a 27-year-old man) who live in the suburbs of Madrid, Spain, were referred to our institution for sneezing, nasal congestion, rhinorrhea, and persistent, dry cough, without dyspnea or wheezing. The symptoms had been present within the last 3 to 4 years, from late December until March (pollination period for *Cupressus arizonica* in Spain), and the patients remained asymptomatic the rest of the year.

Both patients had normal chest radiograph results and negative Mantoux test results. Other common causes of chronic cough, such as gastroesophageal reflux, were ruled out. Skin prick tests with common aeroallergens (ALK-Abelló, Madrid, Spain) and total and specific IgE measurements (Phadia, Uppsala, Sweden) were performed. Spirometry, methacholine challenges, fraction of exhaled nitric oxide (FeNO) measurements (NioxMino; Aero-

crine AB, Solna, Sweden), and sputum induction were performed both during and outside the pollination period.

Both patients were monosensitized (positive skin prick test result and positive specific IgE determinations) to *C arizonica* pollen (2.19 and 1.76 kU/L, respectively). Total serum IgE measurements were 87 and 25 kU/L, respectively. Spirometric parameters were always normal (patient 1: forced vital capacity [FVC], 3.15 L [105%]; forced expiratory volume in 1 second [FEV₁], 3.1 L [119%]; FEV₁/FVC, 98%; patient 2: FVC, 6.15 L [115%]; FEV₁, 4.91 L [115%]; FEV₁/FVC, 79%), and no changes were observed after inhalation of salbutamol, 400 µg (administered via metered-dose inhaler), either during or outside the pollination season. Methacholine challenges, performed using the dosimeter method, produced negative results in February and September (provocative concentration inducing a 20% decrease in FEV₁, >16 mg/mL). Cough frequency score, which was obtained using a visual analog scale (0 = never cough, 10 = cough all day), was correlated with the Cupressaceae pollen load in 2009 (0 score out of season in both patients and 6 and 5 score in patient 1 and patient 2, respectively, during the 2009 *C arizonica* pollen season). The Cupressaceae annual total pollen count during 2009 was 4,500 grains/mm³.

Sputum samples were collected after inhalation of hypertonic saline and then processed following European Respiratory Society recommendations³ within 2 hours after induction, as previously described.⁴

In the second patient, the sputum induction performed in February was unsuccessful, so we performed SIC with *C arizonica* pollen extract (ALK-Abelló) out of its pollination season. A sputum sample was obtained and processed the previous day, as the baseline reference. Twenty-four hours after the SIC, a methacholine test was repeated, followed by a new sputum induction. FeNO measurements were performed before and 24 hours after the SIC, previous to pulmonary function and airway hyperresponsiveness testing. The patient developed dry cough at the end of the challenge, but neither asthmatic response nor change in airway responsiveness to methacholine was observed within the 24 hours after the SIC. The FeNO measurements and the percentage of eosinophils in induced sputum during and outside the *C arizonica* season (patient 1) and before and after the SIC with *C arizonica* extract (patient 2) are summarized in Table 1.

SIC with *C arizonica* pollen extract produced negative results in 2 control nonatopic subjects and in 2 patients with rhinoconjunctivitis who were sensitized to *C arizonica* pollen.

To our knowledge, this is the first report of sensitization to a common pollen (*C arizonica*) as a causative agent of NAEB. The cough totally subsided in both patients after treatment with inhaled corticosteroids (budesonide, 400 µg twice daily) during *C arizonica* pollination season the following year.

Table 1. Comparison of FeNO and Eosinophils in Sputum With or Without Exposure to *Cupressus arizonica* Pollen

	FeNO, ppb		Eosinophils in induced sputum, %	
	Out of season	Exposure to pollen	Out of season	Exposure to pollen
Patient 1	24	68	2.3	33 (season)
Patient 2	14	41	1	8.3 (SIC)

Abbreviations: FeNO, fraction of exhaled nitric oxide; SIC, specific inhalation challenge.