Facial Rash and Palmoplantar Pruritus in an Infant After First Contact With Kaki

A Kitano,[1] T Miyazaki,[2] K Yoshioka,[3] T Kurono,[4] S Kurono,[4] T Matsumoto[3]

[1] *Kitano Children's Clinic, Chikami, Kumamoto, Japan*

[2] *Miyazaki Children's Clinic, Sadowara, Kumamoto, Japan*

[3] *Department of Child Development, Graduate School of Medical Sciences, School of Medicine, Kumamoto University, Kumamoto, Japan*

[4] *Kurume University Translational Research Center, Kurume, Japan*

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Palabras clave: Caqui. Sharon. Fruta de persimonio. Alergia a frutas. Alergia a polen.

Kaki, also known as Sharon fruit or persimmon, is the edible fruit of the persimmon tree (Diospyros kaki), which belongs to the Ebenaceae family. Although kaki has been a very popular food in Japan for centuries, no allergies to this fruit have been previously reported in this country.

A 13-month-old male infant was brought to the outpatient clinic at Kumamoto University Hospital in Kumamoto, Japan with a reaction to kaki upon first ingestion. He exhibited skin rash and itching on the face and soles of the feet. The symptoms had appeared immediately after biting a fresh kaki and the itching on the soles persisted for more than 13 hours despite treatment with oral antihistamines. Neither wheeze nor gastrointestinal symptoms were observed and there were no indications of asthma, eczema, or rhinoconjunctivitis.

A positive skin prick test result was obtained with fresh kaki [1], yielding a wheal measuring 18 mm in diameter compared to 6 mm with 1% histamine solution and 0 mm with normal saline. Although the parents did not consent to an oral provocation test with kaki, the result of the CAP-fluorescence enzyme immunoassay (Phadia Diagnotics, Uppsala, Sweden) analysis (3.29 kU/L) confirmed sensitization to kaki. The patient was also sensitized to *Dermatophagoides farinae* (0.50 kU/L) and *D pteronyssinus* (3.14 kU/L), but not to carrot, apple, kiwi, celery, melon, peach, timothy grass, mugwort, alder, birch, beech, oak, or *Alternaria*. Serum specific-immunoglobulin (Ig) E for rBet v1 or rBet v2 was not detected.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis immunoblotting with kaki extracts revealed bands corresponding to apparent molecular masses of 27 kDa, 30 kDa, 60 kDa, and 70 kDa (Figure). The immunoblotting study with the patient's serum indicated IgE-binding proteins at positions corresponding to molecular masses of approximately 30 kDa and 60 kDa. No bands were detected for sera from 2 control infants with hen egg allergy.

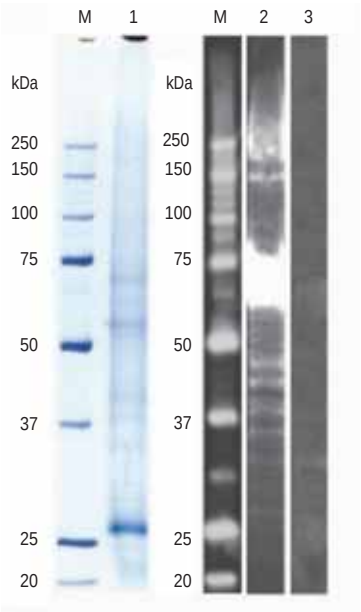

Figure. SDS-PAGE and immunoblotting. M, molecular weight markers; Lane 1, sodium dodecyl sulfate polyacrylamide gel electrophoresis results with kaki extracts after Coomassie staining. Lane 2, immunoblotting with purified human immunoglobulin (Ig) E (Yamasa-Shoyu, Choshi, Chiba, Japan) and alkaline phosphatase-conjugated anti-human IgE (Bethyl Laboratories, Montgomery, Texas,USA). Lane 3, immunoblotting study with kaki extract, patient serum, and alkaline phosphatase-conjugated anti-human IgE.

To the best of our knowledge, this is the first report of an allergic reaction to kaki ingestion in Japan. Seven patients with kaki allergy have been described in the literature, 6 of whom developed an anaphylactic reaction following ingestion [2-5]. Because the infant in our study had not eaten a large amount of kaki, the allergic reaction was limited to dermal sites. Interestingly, palmoplantar itching and erythema have also been documented in 2 of the 7 patients in the literature [2,5].

Anliker et al [2] suggested a role for primary sensitization to grass pollen in kaki allergic patients and implicated profilin (Bet v2) and carbohydrate determinants as the main allergens in kaki. Bolhaar et al [5], in contrast, indicated that birch pollen–related allergens, including Bet v1, might be the primary sensitizers in patients with kaki allergy. The infant in the present study experienced an adverse reaction to kaki upon first contact with this fruit, and there was no evident correlation with pollen or food allergy. The patient's mother had eaten kaki during pregnancy and lactation; because the presence of maternally derived dietary allergen in fetal circulation and breast milk has been confirmed [6], the sensitization might have occurred in utero or in early life.

Only 2 bands, corresponding to approximately 30 kDa and 60 kDa, were detected in the immunoblotting study. Anliker et al [2] also detected a 30-kDa allergen in patients with kaki allergy, reporting that inhibition was possible with kaki extract but not with either Bet v1 or Bet v2. This accumulated evidence suggests

that the 30-kDa allergen might be a pollen-independent kaki allergen. A further hypothesis might be that the 60-kDa allergen is a dimeric form of the 30-kDa kaki allergen.

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Tomoaki Matsumoto
Department of Child Development
Graduate School of Medical Sciences
School of Medicine, Kumamoto University
1-1-1 Honjo, Kumamoto, Japan
E-mail: tomoaki@kumamoto-u.ac.jp

FoxP3$^+$ Regulatory T cells in Childhood Allergic Rhinitis and Asthma

G Mészáros,[1]* B Szalay[1]*, G Toldi[1], G Mezei,[1] L Tamási [2], B Vásárhelyi,[3] E Cserháti,[1] A Treszl[3,4]
[1] *First Department of Pediatrics, Semmelweis University, Bókay u. 53-54, Budapest, Hungary*
[2] *Department of Pulmonology, Semmelweis University, Diósárok u. 1/c, Budapest, Hungary*
[3] *Research Group of Pediatrics and Nephrology, Hungarian Academy of Sciences, Semmelweis University, Bókay u. 53-54, Budapest, Hungary*
[4] *Pediatric Statistic Group of the First Department of Pediatrics, Semmelweis University Bókay u. 53-54, Budapest, Hungary*
* These authors contributed equally to the study.

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Palabras clave: Asma. Severidad. Células T reguladoras.

Introduction

Regulatory CD4$^+$ forkhead box P3 (FoxP3)$^+$ T cells (Tregs) may contribute to asthma and allergy [1] as they possibly favor skewness toward a decreased T_H1/T_H2 ratio [2]. Recent data have emerged showing alterations in Tregs in asthma but the studies reporting these results used a nonspecific cell surface marker (CD25) rather than the obligatory intracellular FoxP3 marker to distinguish Tregs [3-5]. We investigated the prevalence of CD4$^+$FoxP3$^+$ cells and their cellular targets in children with allergic rhinitis and asthma.

We enrolled 22 boys with allergic rhinitis (n=8), allergic rhinitis with mild asthma (n=8), or allergic rhinitis with severe asthma (n=6) and a healthy control group consisting of 13 age-matched boys. The symptoms were evaluated using the Allergic Rhinitis and its Impact on Asthma and Expert Panel Report 3 guidelines [6]. Patients with severe asthma were reassessed 1 month later. Exclusion criteria were the presence of infections, inflammatory diseases other than asthma, and chronic disorders. After parental informed consent was obtained, blood was taken and peripheral blood mononuclear cells isolated and assayed for the markers CD4, CD8, CD25, CD62L, CD69, HLA-DR, and FoxP3 as described previously [8].

The Kruskall-Wallis and Mann-Whitney test were used for comparisons between groups and the Wilcoxon rank sum test to determine changes between 2 different time points in patients with severe asthma. We used the Hettmansperger-Norton nonparametric trend test to analyze the trend between the proportion of Tregs and disease severity.

The proportion of CD4$^+$FoxP3$^+$ cells was comparable in patients and healthy controls, but higher in patients with moderate to severe allergic symptoms than in the control group (P=.027) (Table). Furthermore, our trend analysis revealed an association between symptom severity and the proportion of CD4$^+$FoxP3$^+$ cells (P=.01). No association was observed between symptom severity and Treg cells when the same patients were retested. The proportion of activated (CD25$^+$, CD62L, or HLA-DR) CD4 and CD8 lymphocytes was comparable in each group and the proportion of CD4$^+$FOXP3$^+$ cells was not associated with that of activated lymphocytes or CD4$^+$CD25^{high+} cells.

Previous studies of allergic populations have yielded contradictory results. Based on CD4 and CD25^{high} positivity, 2 studies found lower than normal Treg proportions in children and adults with rhinitis [3,4], whereas another study found a comparable prevalence of CD4$^+$CD25^{high+} Tregs in asthmatic and healthy individuals, but lower Treg numbers in patients with acute asthma than in those with chronic disease [5]. An explanation for these divergent results is that the CD25 surface marker was used to identify Tregs and, as CD25 is an activation marker, CD4$^+$CD25^{high+} cells do not reflect Tregs. Indeed, we were unable to detect an association between CD4$^+$FOXP3$^+$ and CD4$^+$CD25^{high+} cells either.

Our study is the first of its kind to measure FoxP3 protein expression in childhood allergy and data published to date on mRNA levels in asthma have been contradictory [3,4]. Nonetheless, it should be borne in mind that FoxP3 mRNA expression is a poor indicator of FoxP3 protein expression [7], which is why FoxP3 protein rather than mRNA is considered an obligatory marker of CD4$^+$ Tregs.

Table. Frequency of Individual Genotypes in House Dust Mite-Allergic Asthmatics and Healthy Control Subjects[a]

	Allergic Rhinitis (n=8)	Mild Asthma (n=8)	Moderate to Severe Asthma (n=6)		Healthy Controls (n=13)
			Visit 1	Visit 2	
Age, y	11 (8.5-15)	10 (6.8-14)	8.0 (6.3-9.8)		9.0 (7.1-14)
Height, m	1.41 (1.34-1.55)	1.32 (1.23-1.62)	1.28 (1.23-1.40)		1.33 (1.22-11.41)
Weight, kg	35.00 (28.25-55.75)	29.50 (24.75-53.25)	30.00 (26.25-33.00)		34.25 (22.00-51.25)
Proportion of $FoxP3^+$ levels in $CD4^+$ cells, %	2.54 (1.63-3.56)	1.90 (1.07-2.36)	4.18 (3.35-6.27)	3.73 (3.31-4.07)	1.68 (0.88-3.24)
Proportion of $CD4^+$ $CD25^+$ cells, %	5.12 (4.00-9.80)	9.93 (4.87-10.34)	8.35 (2.59-17.08)	7.63 (5.58-10.91)	7.04 (3.79-9.43)
Proportion of $CD4^+$ $CD25^{high+}$ cells, %	2.58 (1.24-12.17)	2.53 (0.97-6.31)	2.54 (0.80-3.84)	2.07 (1.99-3.47)	2.33 (0.71-6.32)
Proportion of $CD8^+$ $CD25^+$ cells, %	0.66 (0.48-0.75)	0.79 (0.45-1.56)	0.84 (0.62-1.01)	0.95 (0.65-1.17)	1.98 (1.29-5.05)
Proportion of $CD4^+$ $CD6L2^+$ cells, %	45.61 (30.79-53.33)	50.53 (30.99-63.02)	24.69 (11.53-36.30)	58.24 (38.87-67.09)	13.62 (12.77-30.83)
Proportion of $CD8^+$ $CD62L^+$ cells, %	34.46 (19.72-36.66)	40.63 (24.05-52.98)	19.65 (7.67-41.07)	48.62 (28.67-55.44)	26.36 (10.22-52.73)
Proportion of $CD4^+$/HLA-DR^+ cells, %	5.43 (4.00-6.15)	4.74 (4.11-7.46)	4.55 (3.06-8.22)	3.72 (3.66-3.76)	7.23 (5.44-9.73)
Proportion of $CD8^+$/ HLA-DR^+ cells, %	4.12 (3.67-9.78)	3.79 (2.49-5.56)	10.41 (5.33-18.93)	3.17 (1.92-4.62)	5.72 (3.83-7.80)

[a] Data are expressed as medians (interquartile range).
[b] $P<.05$.

We also found an association between the proportion of Tregs and severity of disease, possibly supporting a role of Tregs in disease development.

Interestingly, we were unable to detect any association between $FOXP3^+$ positivity and the lymphocyte activation markers we investigated, among them CD25, CD62L, and HLA-DR. Huang et al [8] found no difference between asthmatic patients and control individuals in terms of CD62L positivity. Similarly, we observed no association between disease severity and $CD62L^+$ cell prevalence in this study.

In summary, we found an increased prevalence of $CD4^+FoxP3^+$ Treg cells in children with allergic asthma. Due to the small size of our sample, our work should be considered a hypothesis-generating rather than a confirmatory study.

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Andras Treszl
Research Group of Pediatrics and Nephrology
Hungarian Academy of Sciences
Bókay u. 54, Budapest 1083
Hungary
E-mail: treszl@gyer1.sote.hu

Systemic Reactions in Anamnestic Responses During Penicillin Allergy Study

M Gallego Segovia,[1] AM García Dumpiérrez[2]
[1] *Allergy Service, University Hospital INSULAR Materno-Infantil, Las Palmas de Gran Canaria, Spain*
[2] *Allergy Service, University Hospital of Gran Canaria Dr. Negrin, Sergio Del Pino Pérez Ventura, Health Centre Moya, Las Palmas de Gran Canaria, Spain*

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Palabras clave: Respuesta anamnésica. Anafilaxia. Alergia a penicilina. Tests cutáneos.

A Colombian female aged 41 years, with previously diagnosed stable dust mite–asthma, experienced generalized urticaria and presyncope 15 minutes after the administration of an intravenous infusion of penicillin to treat a respiratory infection. The patient required emergency treatment and the situation resolved within 2 to 3 hours. This reaction occurred 6 years before the penicillin allergy study described below.

Absence of previous use of inhibitory wheal reaction drugs was confirmed. Skin prick tests with penicilloyl-polylysine (PPL), minor determinant mix (MDM), benzylpenicillin (10000 IU/mL), ampicillin (25 mg/mL), amoxicillin (25 mg/mL), cefazolin (25 mg/mL), and saline solution used as a negative control were all negative. Histamine used as a positive control produced a wheal with a 5-mm diameter. Intradermal allergy tests with PPL, MDM, benzylpenicillin (10000 IU/mL), ampicillin (2.5 mg/mL), amoxicillin (2.5 mg/mL), and cefazolin (2.5 mg/mL) were also negative upon immediate evaluation.

A single-blind placebo-controlled oral challenge test with oral penicillin (400 mg) produced epigastric pain 15 minutes post challenge. The pain resolved 20 minutes later with oral omeprazole (20 mg) and the patient was discharged 2 hours later. The test was considered negative. The same protocol was carried out in the patient 15 days later, with confirmation of absence of previous use of inhibitory wheal reaction drugs.

Prick tests with PPL and cefalozin were negative but produced wheals for MDM (diameter, 12 mm), benzylpenicillin (25 mm plus pseudopods), ampicillin (16 mm), and amoxicillin (12 mm). Fifteen minutes after the prick tests, the patient manifested rhinoconjunctivitis, cough, and wheezing. Subcutaneous epinephrine (0.3 cc), nebulized salbutamol (1.5 cc), and an intravenous infusion of methylprednisolone (80 mg) were administered, with symptomatic relief occurring within 30 minutes.

Total serum immunoglobulin (Ig) E levels were 591 kU/L. Specific IgE to benzylpenicillin, penicillin V, cefazolin, ampicillin, and amoxicillin were less than 0.35 kU/L (class 0) 3 weeks before oral provocation. The levels after the reaction were as follows: benzylpenicillin, 2.37 kU/L (class 2); penicillin V, 7.87 kU/L (class 3); ampicillin, 2.96 kU/L (class 2); amoxicillin and cefazolin, <0.35 kU/L (class 0). Serum tryptase levels were 6.76 μg/L at baseline, 6.00 μg/L 10 minutes after the reaction, and 5.19 μg/L 30 minutes after the reaction. Skin prick tests, intradermal skin tests, and single-blind placebo-controlled challenges with intramuscular ceftazidime (250 mg) and oral cefuroxime (250 mg) were negative.

Anaphylaxis to penicillin was diagnosed. IgE-mediated hypersensitivity to penicillin may be considered an anamnestic response when there is a previous, documented reaction. If the initial study is considered negative, further evaluation is mandatory [1,2]. The time period between 2 studies is not firmly established, although a minimum interval of 2 weeks seems to be reasonable [3,4]. There are no data available on the prevalence of anamnestic responses during penicillin allergy studies.

The above protocol has been applied in the diagnosis of ß-lactam IgE hypersensitivity at our hospital for 15 years. Of the 2065 patients studied in this time, only 145 (0.07%) have shown a positive response. The prevalence of anamnestic responses is 1 in 145 patients with penicillin allergy (0.007%)

and 1 in 2065 patients evaluated for penicillin allergy (0.005 per thousand). The risk of anaphylactic reactions induced by a penicillin prick test is less than 0.02%-0.049% [5] and these reactions are more common in subjects with previous anaphylactic reactions, and particularly when penicillin and amoxycillin are implicated [4].

Skin and respiratory reactions are most common and usually improve with adequate treatment [6]. In our case, the patient's respiratory symptoms responded well to epinephrine, salbutamol, and corticosteroids. Our results confirm that immediate hypersensitivity to ß-lactam studies must be carried out in duplicate in order to confirm an anamnestic response.

High sensitivity, low cost, and immediate results make skin tests most suitable for diagnosing IgE-mediated reactions to ß-lactams. Because anaphylactic reactions are rare, these tests are considered a safe diagnostic tool [7].

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Antonio Manuel García Dumpiérrez
Servicio de Alergia
Hospital Universitario de Gran Canaria Dr. Negrín
Barranco de La Ballena s/n. 35010
Las Palmas de Gran Canaria
Spain
E-mail:sarabgd@hotmail.com

False Positive Placebo Reaction in a Double-Blind Placebo-Controlled Food Challenge

I Reig Rincón de Arellano,[1] S Vázquez-Cortés,[1]
AC Sinaniotis,[2] M Fernández-Rivas[1]

[1] *Servicio de Alergia, Hospital Clinico San Carlos, Madrid, Spain*
[2] *Allergy Research Centre, Second Department of Pediatrics, P&A Kyriakou Children's Hospital, University of Athens, Greece*

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Palabras clave: Alergia. Diagnóstico. Provocación controlada doble ciego con placebo frente a alimentos. Alergia alimentaria.

Food allergy diagnostic tests include skin prick tests (SPTs), specific immunoglobulin (Ig) E antibody determinations, and food challenges [1]. Since double-blind placebo-controlled food challenges (DBPCFCs) are the gold standard in the diagnosis of food allergy, clinicians should be extremely careful when performing them to avoid misleading results. Relatively little attention has been paid to the issue of false negative and positive responses in DBPCFCs until recently [2-4]. As there are often other people such as relatives, visitors, and other patients in the ward where patients undergo DBPCFCs, inadvertent exposure to the allergen being tested during a placebo challenge may occur, resulting in a false positive reaction. We present a case that illustrates such a situation.

A 15-month-old boy with a previous history of atopic dermatitis was referred to our allergy department for evaluation of an immediate reaction to eating a boiled egg. The reaction consisted of generalized urticaria and facial and scrotal edema. The allergological study was carried out with the parents' consent and within the context of the European Union–funded project EuroPrevall. SPTs with egg yolk and white, ovomucoid, and ovalbumin (ALK-Abelló S.A.,Madrid, Spain) were all positive (mean wheal diameter, 4-6 mm). Specific IgE was negative for egg yolk and ovomucoid, and positive for egg white (0.59 kU/L) and ovalbumin (0.71 kU/L) (ImmunoCAP; Phadia, Uppsala, Sweden). The patient underwent a DBPCFC with milk formula as placebo food and diluted freeze-dried egg in the formula as active food. Active and placebo were given at random on 2 separate days. The protocol comprised 9 doses with a protein content ranging from 3 µg to 3 g that were given at 20-minute intervals. Objective reactions were observed on both challenge days. On the active day (revealed on opening the code), 12 minutes after the intake of 300 mg of egg protein, the child developed perioral wheals and generalized urticaria and vomited twice. The reaction subsided with treatment including epinephrine. During the placebo challenge, the child developed erythema and wheals on the cheeks, perioral erythema, and eye redness 2 hours after taking the complete set of doses. He was given oral corticosteroids and antihistamines, and the reaction subsided in less than 30 minutes. When the outcome of the DBPCFC was discussed with the child's parents, the

father mentioned that on the placebo day he had eaten an omelet sandwich less than half an hour before the onset of the child's reaction; immediately afterwards he had played with and kissed his son without washing his hands or mouth. With this information, we suspected that the patient had experienced contact urticaria caused by egg allergen transferred by the father. The placebo challenge was repeated 1 week later and was completely negative.

This case clearly shows how a positive reaction during a placebo challenge can be caused by the inadvertent transfer of allergen from another party. It is therefore of great importance to advise the relatives of patients with food allergies not to ingest the food under study while the challenges are being performed to avoid false positive reactions. Furthermore, staff preparing challenge meals should be made aware of the risk of cross-contamination via hands, kitchen utensils, clothes, etc and advised to be extremely careful during the entire procedure. Children on the same ward can also inadvertently transfer allergens to each other during challenge observation periods. All these practical aspects of oral food challenges should be taken into account not only to improve test reliability but also to increase patient safety.

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Montserrat Fernández Rivas
Hospital Clínico San Carlos
Servicio de Alergia
Prof. Martín Lagos s/n
28040 Madrid
Spain

Occupational Allergy to Fungal Lipase in the Pharmaceutical Industry

G Loureiro,[1] B Tavares,[1] C Pereira,[1] M Lundberg,[2] C Chieira[1]

[1] *Immunoallergy Department, Coimbra University Hospitals, Coimbra, Portugal*
[2] *MIAB, Uppsala, Sweden*

Key words: Enzyme allergy. Fungal lipase. Occupational allergy. Occupational asthma. Occupational rhinoconjuntivitis.

Palabras clave: Alergia a enzimas. Lipasa fúngica. Alergia ocupacional. Asma ocupacional. Rinoconjuntivitis ocupacional.

Occupational allergy to lipase has been reported in the detergent industry [1-4]. While the main allergenic enzyme in the pharmaceutical industry is amylase, there have been reports of lipase sensitization, albeit without clinical relevance [5,6].

We report the case of a 46-year-old nonsmoking man with allergic rhinoconjunctivitis to grasses since the age of 34 years who had been working in the pharmaceutical manufacturing industry for 25 years. Five years prior to evaluation by our department, the patient started to exhibit rhinoconjunctivitis symptoms and dyspnea at the workplace while handling pancreatic enzyme preparation (PEP) tablets. The medication included fungal lipase (60 000 FIP units/g) derived from *Rhizopus oryzae* (American Laboratories Incorporated, Omaha, New England, USA), fungal amylase derived from *Aspergillus oryzae* (Amano Enzyme Incorporated, Nakaku, Nagoya, Japan), and pepsin. The symptoms started 3 hours after the patient first handled the tablets, worsened throughout the day, and improved after work. The patient did not experience symptoms out of work, during the weekend, during holidays, or at the workplace when PEP was not being manufactured.

Total serum immunoglobulin (Ig) E was 124 IU/mL; skin prick tests (SPTs) with commercial extracts of common aeroallergens, including molds and latex (ALK Abelló, Madrid, Spain) were positive for grass pollen but negative for Aspergillus oryzae amylase commercial extract (Leti, Madrid, Spain) and for substances handled during the manufacture of pharmaceutical products at the workplace, among them *Aspergillus oryzae amylase* and *Rhizopus oryzae* lipase (10% dilution in NaCL 0.9%). Serum specific IgE levels (ImmunoCAP; Phadia, Uppsala, Sweden) were 8.1 kU/L for *Dactylis glomerata*, 7.3 kU/L for *Festuca elatior*, 8.6 kU/L for *Lolium perenne*, 6.9 kU/L for *Phleum pratense*, 7.6 kU/L for *Poa pratensis*, and <0.35 kU/L for nAsp o 1 α-amylase. Skin patch tests with the European standard battery (Chemotechnique Diagnostics, Malmö, Sweden) were positive to neomycin sulphate and mercury ammonium chloride. Patch tests with occupational substances (10% in petrolatum) including PEP fungal enzymes were positive to fungal lipase. Baseline lung function tests showed reversible small

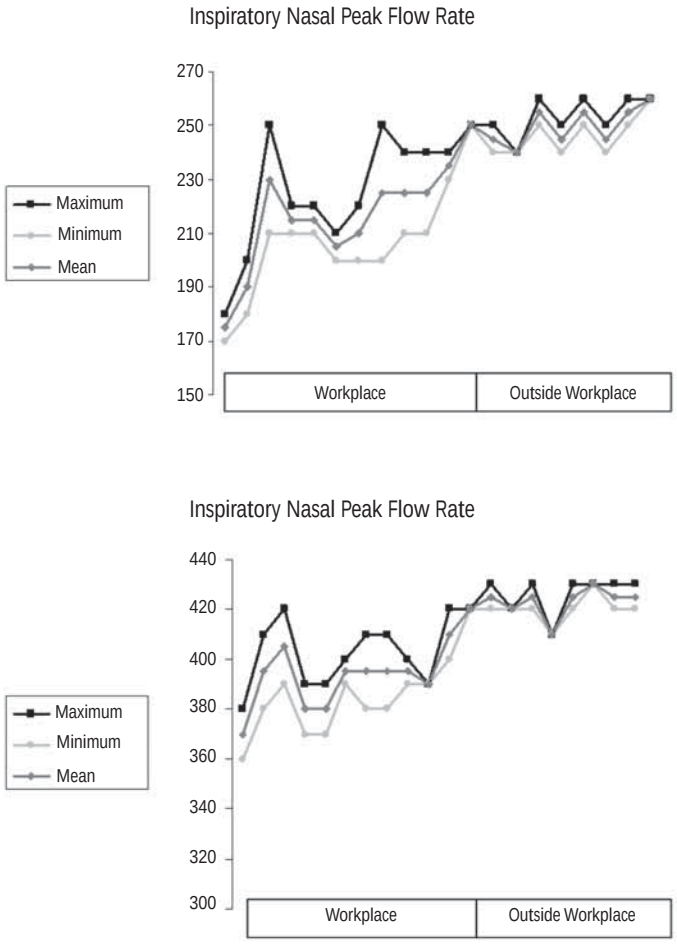

Figure. Inspiratory nasal peak flow rates and peak flow rate monitoring, 10 days at the workplace and 10 days outside the workplace.

airways obstruction (forced vital capacity, 4.27 L [110% of predicted]; forced expiratory volume in 1 second, 3.13L [97% of predicted], forced expiratory flow [FEF]$_{25\%-75\%}$, 2L/s [50% of predicted], 25% bronchodilator reversibility in FEF$_{25\%-75\%}$). A methacholine inhalation challenge test was positive (PC20 at 0.36 mg) when the patient had been at work for 2 weeks but negative when he had been off work for the same time. The 2 challenges were performed outside the grass pollen season.

Monitoring of nasal inspiratory peak flow (NIPF) and peak expiratory flow (PEF) in and outside the workplace showed a worsening of lung and nasal function at work, suggesting that the respiratory symptoms had an occupational origin [7]. The Figure shows the maximum, minimum, and median NIPF and PEF values. The daily variability in NIPF and PEF was greater when the patient was at work (10%-50% for NIPF and ≥20% for PEF) than when he was not (≤10% for NIPF and PEF). A specific nasal provocation test (SNPT) [8] yielded a positive symptom score.

Despite the negative SPT result for lipase, we decided to proceed with further investigation. Using an experimental ImmunoCAP test (Phadia), we detected serum specific IgE levels to fungal lipase of 4.5 KU/L.

A coworker who presented similar symptoms to those experienced by our patient during PEP handling tested positive to α-amylase and negative to lipase during skin prick and patch testing with the same series of occupational allergens as those used in our patient. The same tests carried out in 2 healthy subjects were negative to all extracts, as was an SNPT performed in a healthy worker.

The occupational origin of the respiratory symptoms experienced by our patient was evidenced by the worsening of respiratory function during exposure to PEP at the workplace. Sensitization to fungal lipase was confirmed on observing increased serum specific IgE levels and positive patch test and SNPT results. While occupational respiratory allergies caused by fungal enzymes are described in the literature [1-4], to the best of our knowledge, this is the first report of fungal lipase allergy in a patient not sensitized to amylase working in the pharmaceutical industry. The serum specific IgE and SNPT results and the delayed-type cutaneous reactivity pattern to lipase all suggest the involvement of IgE-mediated and cell-mediated mechanisms.

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Graça Loureiro
Serviço de Imunoalergologia
Hospitais da Universidade de Coimbra
Apartado 9057
Praceta Mota Pinto
3001-301 Coimbra
Portugal
E-mail: gracamloureiro@hotmail.com

Selective Allergy to Raw Pork

J Domínguez-Ortega,[1] B Rodríguez-Jiménez,[1] A Ledesma,[1] C Kindelan,[2] JM González,[2] F Jiménez[3]

[1]*Allergy Unit, Hospital Universitario de Getafe, Getafe, Spain*
[2]*ALK-Abelló, Madrid, Spain*
[3]*Pediatric Department, Hospital del Tajo, Aranjuez, Spain*

Key words: Raw pork. Meat. Allergy.

Palabras clave: Carne cruda de cerdo. Carnes. Alergia.

Although meat allergies are rare, there are an increasing number of case reports, varying from oral allergy syndrome, skin involvement, bronchospasm, and even anaphylaxis, especially with regard to beef [1], to reactions following ingestion and inhalation of, or contact with, cattle, lamb, and horse meat. In many cases, an immunoglobulin (Ig) E–mediated immune mechanism was demonstrated. Pork allergy is less common, especially when it is not associated with allergy to meat from other mammals [2] or with the so-called pork-cat syndrome, where patients sensitized to cat dander develop symptoms after ingesting pork [3].

A 6-year-old child presented oral pruritus, perioral erythema, and mild labial angioedema every time he ate fresh and vacuum-packed cured ham. The reactions became increasingly severe (with onset a few minutes after ingestion), to the extent that he required antihistamines to control symptoms. He also presented symptoms when eating other cold meats such as pork loin, homemade chorizo, and *fuet* (cured sausage). He tolerated boiled ham, fried pork, beef, and lamb. The reactions occurred without exercise, and there was no history of reactions to food or drugs. Neither the patient nor his first-degree relatives had a history of atopic allergy.

Skin-prick-tests were performed with commercial extracts of pollen, profilin, Pru p 3, molds, dog, cat, horse and cow danders, mites, latex, and foods including milk, egg, meats, spices (ALK-Abelló, Madrid, Spain; LETI, Barcelona, Spain), and bovine serum albumin (Diater, Madrid, Spain). They were negative to all the allergens tested except commercial raw pork extract. Skin prick test results were positive to raw pork and negative to cooked pork, and raw and cooked beef.

Total-IgE (CAP system) was 87 kU_A/L and no specific IgE values above 0.35 kU_A/L to beef, cat dander, or bovine serum albumin were detected.

A boiled pork extract was prepared by boiling raw pork at 100ºC for 10 minutes and extracted with a magnetic stirrer at 10% (weight/volume) in a phosphate buffer. Afterwards, it was centrifuged and filtered through 0.8-, 0.45-, and 0.22-mm membranes and saved in aliquots at –20ºC. Likewise, a raw meat extract was analyzed (ALK-Abelló-EC-batch-U190). The two extracts and the molecular weight markers were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (16% acrylamide concentration) under nonreducing conditions. Proteins were then electrophoretically transferred onto NC papers [4], saturated with 0.2% Tween 20 in PBS, and incubated with the patient's serum diluted 1:5 for 18 hours. They were incubated with human anti-IgE monoclonal-antibody HE-2 (1:3000), and, after washing again, they were incubated with peroxidase-conjugated rabbit-antimouse-IgG diluted to 1:5000. Finally, proteins with IgE-binding capacity were detected by means of chemiluminescence.

As the Figure shows, the IgE in the patient's serum recognized a protein band of about 60 kDa in the raw pork extract, and this could coincide with the molecular weight of albumin. However, the patient's serum did not recognize any bands in the cooked meat extract. In the negative control, nonspecific binding was detected, but this did not coincide in intensity or in molecular weight with the band recognized by the patient's serum.

This is the first report of allergy to raw pork to demonstrate an IgE-mediated mechanism by identifying the allergenic

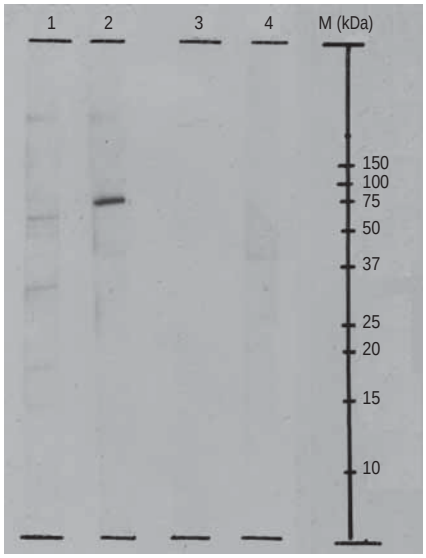

Figure. Results of IgE immunodetection. Lane 1, raw pork extract and negative control; lane 2, raw pork extract and the patient's serum; lane 3, cooked meat extract and negative control; lane 4, cooked meat extract and the patient's serum.

protein. One similar case [5] also used skin tests, but the protein involved could not be identified. In our case, the in vitro study results suggest that serum albumin might be responsible for this patient's clinical symptoms. Nevertheless, this hypothesis has not been totally proven, as an inhibition test with pork albumin was not performed. We also showed that the protein identified in our case was heat-labile, thus enabling it to be destroyed during the meat boiling process, and pork to be tolerated.

Acknowledgments

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Dr Javier Domínguez-Ortega
Servicio de Alergología,
Hospital Universitario de Getafe
Carretera de Toledo, Km 12,500
28905 Getafe (Madrid), Spain
E-mail: alergologia.hugf@salud.madrid.org

Anaphylactic Shock Caused by a 33-kDa Alpha S1-Casein–Like Allergen in Kingfish Caviar

YH Chen,[1,2] HJ Wu,[1] JJ Tsai,[3] MF Lee[3]

[1]*Division of Allergy, Immunology and Rheumatology, Taichung Veterans General Hospital, Taichung, Taiwan*
[2]*National Yang-Ming University, Taipei, Taiwan*
[3]*Department of Education and Research, Taichung Veterans General Hospital, Taichung, Taiwan*

Key words: Anaphylaxis. Caviar. Roe. Allergen. Casein-like protein.

Palabras clave: Anafilaxia. Caviar. Huevas. Alérgeno. Proteína similar a la caseína.

The frequency of allergic reactions to food, particularly shellfish, is increasing among Taiwanese adults (YH Chen, unpublished data). Kingfish *(Scomberomorus commerson*) is widely consumed in Taiwan, both for its meat and its roe. Roe is an extremely rare cause of anaphylaxis and only 1 case of Russian Beluga caviar–induced anaphylaxis and 1 case of salmon caviar–induced anaphylactic shock have been reported in the literature [1,2]. The allergen has never been identified.

We report a 42-year-old woman who experienced itching wheals, flushing, abdominal cramps, bronchospasm, and shock approximately 30 minutes after ingesting kingfish caviar salad. She developed acute respiratory failure and profound shock in the emergency department, and required intubation and temporary mechanical ventilatory support. She recovered the following day with no significant complications. She had been diagnosed with allergic rhinitis in her teens and had a 4-year history of optimally controlled bronchial asthma. During the past 10 years, she had experienced acute urticaria after consuming shrimp, but did not recall any problems related to fish, milk, or chicken egg.

Crude extract of kingfish caviar was made from the same dish that the patient ingested with phosphate buffered saline as described elsewhere [3]. A skin prick test performed 2 weeks later showed positive reactions to in-house–produced crude kingfish caviar extract (200 μg/mL), commercial house dust mite, and shrimp, but no reaction to milk, egg yolk, egg white, cat dander, dog dander, grass, weed, trees, *Aspergillus* species, *Penicillium* species, *Cladosporium* species, *Candida albicans* (Greer Laboratories, Lenoir, North Carolina, USA), or latex (Stallergenes, Antony, France). Crude kingfish caviar proteins were then separated using 4-12% sodium dodecylsulfate polyacrylamide gel electropheresis (SDS-PAGE) and immunoblotted with sera from the patient and a nonallergic control at a 1:9 dilution. Immunoblotting was performed using anti-human immunoglobulin (Ig) E alkaline phosphate conjugate and 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium substrate. The patient's serum recognized 2 IgE-binding proteins with molecular weights of 33 kDa and 43 kDa, (lane P in panel B of the Figure), whereas no reactivity was detected for the nonallergic control (lane N). After 2-dimensional (2-D) PAGE and immunoblotting as described above (except for the use of a chemiluminescent substrate solution) (panel C), the 33-kDa target protein was excised for in-gel trypsin digestion using silver-stained 2-D PAGE, analyzed by electrospray ionization tandem mass spectrometry (Finnigan MAT, San Jose, California, USA), and searched with a Mascot server (Matrix Science, London, UK). The 33-kDa protein showed significant homology with the alpha S1-casein of *Bos taurus* (domestic cow) at the following sequences: ^{23}HQGLPQEVLNENLLR^{37}, ^{106}YLGYLEQLIR^{115}, and ^{148}EPMIGVNQELAYFYPELFR^{166} (GI:162794, score 151). The 43-kDa IgE-binding protein observed in lane P was not detected on 2-D PAGE.

Alpha-S1-casein is a well-known major allergen of cow's milk [4], but has never been reported as an allergen of fish or caviar. There have been reports of casein used as an ingredient of synthetic caviar [5], and 1 case report described anaphylaxis caused by the unexpected presence of casein in reconstructed salmon in a patient who was allergic to milk [6]. However, our patient was not allergic to milk, and the caviar she consumed was a gift from a fisherman and was poached just before

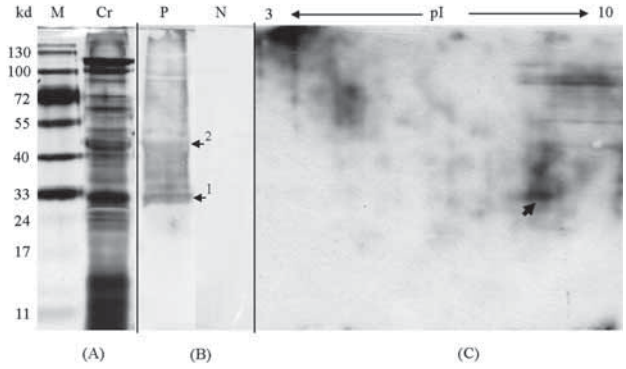

Figure. Panel A, Protein patterns by Coomassie brilliant blue-stained SDS-PAGE: lane M, molecular markers; lane Cr, crude kingfish extract. Panel B, Immunoblotting with the patient's serum (lane P) revealed 2 IgE-binding proteins at 33 kDa and 43 kDa (arrowhead 1 and 2). No IgE binding band was detected using the serum of the nonallergic control (lane N). Panel C, 2-D gel electrophoresis and immunoblotting revealed strong IgE-binding activity at the 33-kDa protein (arrowhead) with an estimated pI of 7.2. Ig indicates immunoglobulin; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis.

consumption, thus ruling out the possibility of casein-based "fake" caviar or cow milk contamination during cooking.

The immunoblotting data and skin test results allow us to conclude that our patient had IgE-mediated anaphylactic shock caused by a 33-kDa casein-like allergen in kingfish caviar. Clinicians should be aware of uncommon food allergens in order to prevent fatal allergic reactions.

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Dr MF Lee
Department of Education and Research
Taichung Veterans General Hospital
Taichung, Taiwan
E-mail: mflee@vghtc.gov.tw

Successful Adalimumab Desensitization After Generalized Urticaria and Rhinitis

B Rodríguez-Jiménez,[1] J Domínguez-Ortega,[1] C González-Herrada,[2] C Kindelan-Recarte,[1] P Loribo-Bueno,[1] N Garrido-Peño[3]
[1]*Allergy Unit, Hospital Universitario de Getafe, Getafe, Spain*
[2]*Dermatology Service, Hospital Universitario de Getafe, Getafe, Spain*
[3]*Pharmacy Service, Hospital Universitario de Getafe, Getafe, Spain*

Key words: Adalimumab. Desensitization. Drug allergy. Psoriasis. Tumor necrosis factor-□ antagonists.

Palabras clave: Adalimumab. Desensibilización. Alergia a fármacos. Psoriasis. Antagonistas del factor de necrosis tumoral-alfa.

Adalimumab (Humira) is a recombinant human monoclonal antibody that inhibits tumor necrosis factor □ (TNF-□). It is used for the treatment of rheumatoid arthritis, ankylosing spondylitis, Crohn disease, psoriatic arthritis, and moderate-to-severe chronic plaque psoriasis. The recommended dosage for adults with psoriasis is an initial subcutaneous dose of 80 mg followed by 40 mg administered every other week, beginning 1 week after the initial dose. Allergic reactions to adalimumab appear in approximately 1% of patients according to clinical studies [1].

We report the case of a 42-year-old woman monitored by our dermatology department for extensive plaque psoriasis since the age of 6 years. She had shown a poor response to several treatments. In recent years, the patient had been treated with methotrexate and etanercept with no improvement in her skin lesions. In 2007, she began therapy with infliximab, presenting regular tachycardia with a narrow QRS complex at 1 year of treatment. The medication was discontinued and replaced by adalimumab. After the first doses, the plaques disappeared almost completely. Nevertheless, after the third dose, the patient developed a wheal with edema at the injection site. The wheal resolved without treatment after a few hours. Within a few minutes of the sixth injection of adalimumab, the patient presented nasal obstruction, generalized itching, and urticaria. She required treatment with intramuscular corticosteroids and antihistamines. Adalimumab was discontinued, and the psoriasis deteriorated.

Skin prick tests with adalimumab (50 mg/mL) elicited a positive response, although patch tests with adalimumab 5% and 10% in sterile water were negative. Skin prick tests with adalimumab were negative in 10 controls.

Given the success of adalimumab and the lack of response to other drugs, the patient agreed to be re-treated with adalimumab using a desensitization protocol. Desensitization was carried out in the outpatient clinic of our allergy department. The patient did not receive any premedication. After she signed an informed consent form, the desensitization protocol began with an initial subcutaneous dose of 0.5 mg

Table. Protocol for Induction of Tolerance to Adalimumab

Dose	Concentration	Volume, mL[a]	Cumulative Dose, mg
1	1/100	1	0.5
2	1/10	0.25	1.75
3	1/10	0.5	3.25
4	1/1	0.1	8.25
5	1/1	0.2	18.25
6	1/1	0.5	44.25

[a] Each dose was completed with sterile water until 1 mL.

(1/100) that was gradually increased until a cumulative dose of 44.25 mg was reached (Table). The intervals between doses were approximately 60 minutes. About 45-60 minutes after the second and third doses (1.25 and 2.5 mg, respectively), the patient presented an injection site reaction, consisting of skin eruption, edema, and itching. She required no treatment and was able to continue the regimen until the total dose was reached. The complete process took 6 hours.

The patient was treated with subcutaneous adalimumab every other week following our desensitization protocol without incident. She experienced a significant clinical improvement over the following weeks.

The use of biological agents is increasing. Despite their clinical utility, they are associated with hypersensitivity reactions [2]. In patients undergoing therapy with adalimumab, there have been reports of injection site reaction [3], urticaria [4,5], an erythema multiforme–like reaction in a patient with rheumatoid arthritis [6], pustular eruption in flexural areas [7], and even the development of psoriasiform lesions [8].

In patients with IgE-mediated reactions to biological agents, the induction of tolerance might be an acceptable option. Desensitization protocols have been carried out with other biological agents, such as infliximab, achieving therapeutic doses in patients who had presented anaphylactic or anaphylactoid reactions during treatment [9]. However, to date, no guidelines for adalimumab desensitization have been published.

We report the first case of generalized urticaria and rhinitis after therapy with adalimumab. The IgE-mediated mechanism of the reaction was confirmed by skin tests. A subcutaneous desensitization protocol was completed successfully in this patient.

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Beatriz Rodríguez Jiménez
Allergy Unit
Hospital Universitario de Getafe
Carretera de Toledo, Km 12,500
28905 Getafe (Madrid), Spain
E-mail: alergologia.hugf@salud.madrid.org

Should the Skin Prick Test to Horse Be Included in the Standard Panel for the Diagnosis of Respiratory Allergy?

E Novembre, F Mori, S Barni, N Pucci, ME Rossi
Allergy Unit, Azienda Ospedaliero-Universitaria A Meyer Florence, Italy

Key words: Children. Horse allergy. Respiratory allergy. Skin prick tests.

Palabras clave: Niños. Alergia a caballo. Alergia respiratoria.

Horse allergy has received little attention in the literature, although it affects both pediatric and adult patients, and is considered a risk factor for developing asthma, rhinitis, conjunctivitis, and eczema [1]. The prevalence of horse allergy is extremely variable (from 1% to 10%), depending on geography, climate, and level of exposure [1-3]; however, few studies examine specific patient groups. We performed a retrospective study of horse allergy and reported the prevalence of pediatric cases referred to our allergy unit.

Over the last 8 years, almost all children (age 2-16 years) attending our unit underwent skin prick testing for the common aeroallergens: *Dermatophagoides pteronyssinus*

Table. Prevalence of Symptoms in 184 Children Sensitized to Horse Allergen

Children Sensitized to Horse	No Symptoms	Rhinitis	Asthma	Urticaria	Urticaria and Rhinitis
No contact	80 (43.5%)				
Close to horses with no direct contact		32 (50%)	16 (25%)	8 (12.5%)	8 (12.5%)
Contact with horses	40 (20%)				

and *Dermatophagoides farinae*; cat, dog and horse dander; *Alternaria tenuis* and *Cladosporium*; grass, cypress, *Olea europaea*, birch, plane tree, *Carpinus, Parietaria*, and *Artemisia.*

Skin prick tests with commercial extracts (Alk-Abelló, Milan, Italy; Bayer, Milan, Italy) were performed on the volar aspect of the forearm using metal lancets according to international guidelines [4].

One hundred eighty-four children were tested during the last year. Telephone interviews were conducted to determine whether they had ever been in contact with horses and/or been in places such as a racetrack or a stable, and whether they had experienced an allergic reaction after proximity to or contact with horses (Table).

The prevalence of horse sensitization was 2.7% (624 positive results) in a population of 23,460 children who underwent skin prick testing and the following results were obtained: *D pteronyssinus*, (28.7%), *D farinae* (26.4%), cat (15%), dog (5.0%), hamster (0.6%), *Alternaria* (5.0%), *Cladosporium* (0.8%), grass (33.2%), *Parietaria* (5.2%), *Artemisia* (5.6%), *Olea europaea* (12.8%), birch (8.3%), *Carpinus* (8.0%), plane tree (5.0%), oak tree (0.5%), cypress (4.1%), and oak chestnut (3.0%).

The prevalence of horse sensitization is extremely variable in children, and patients with no history of allergy or contact with horses can be affected [5,6]. In addition to being a risk factor for asthma, rhinitis, conjunctivitis, and eczema [1], sensitization to this allergen has been reported to be a risk factor for severe reactions. There have been reports of anaphylaxis caused by contact with horses or bites [7,8], and a fatal reaction during physical exercise carried out immediately after a visit to a stable [9].

Sensitization to horse allergen is easily determined using the skin prick test, although the inclusion of horse allergen in the routine workup for diagnosis of respiratory allergy is controversial.

Heinzerling and coworkers [10] compared skin prick test procedures performed in 29 allergy units from the Global Allergy and Asthma European Network in Europe. Different panels were used, although horse allergen was tested in only 10 (34%) of the 29 units. The authors concluded that routine testing for that allergen was not useful when the personal history was negative. The presence of horse allergen in Swedish schools has been significantly correlated with wheeze, daytime breathlessness, and asthma [11]. Our results revealed a 2.7% prevalence of allergy to horse in a population of 23 460 pediatric patients attended at an allergy unit. Although this prevalence is low, we recommend including horse extract in the routine panel, taking into account the following factors: 1) horse sensitization is often symptomatic and may lead to severe allergic reactions [3-7]; 2) horse allergy can develop without previous close contact [5]; and 3) horse allergen can be carried on clothes, and therefore sensitize people who have absolutely no contact with horses [6].

In conclusion, we recommend inclusion of horse allergen in the routine panel, not only to verify a suspected allergy before treating it, but also to inform patients who are unaware of their allergy of the risks connected with exposure to high amounts of the allergen.

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Dr Francesca Mori
Allergy and Clinical Immunology Unit
Department of Pediatrics
University of Florence
Viale Pieraccini 24,
50139 Florence, Italy
E-mail: francymori@libero.it

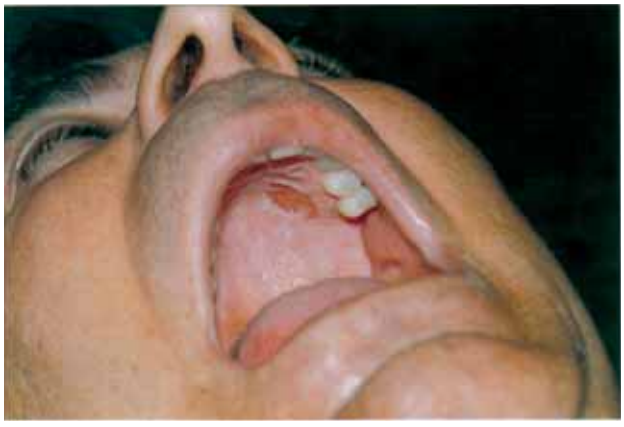

Figure. Enanthema on the roof of the mouth.

Enanthema and Fixed Drug Eruption Caused by Trimethoprim

MC Moreno Escobosa, S Cruz Granados, MC Moya Quesada, J Amat López
Allergy Department, Hospital Torrecárdenas, Almería, Spain

Key words: Cotrimoxazole. Enanthema. Fixed drug eruption. Trimethoprim.

Palabras clave: Cotrimoxazol. Enantema. Exantema fijo. Trimethoprin.

Trimethoprim is a diaminopyrimidine derivative that inhibits bacterial growth by interfering with the synthesis of folic acid. This antibacterial drug has been available as a single agent since 1979, and is indicated mainly for the treatment of urinary tract infection. Trimethoprim is widely used in combination with sulfamethoxazole (cotrimoxazole). This drug combination has synergistic bactericidal activity against gram-negative and gram-positive organisms and is widely used. Sulfamethoxazole is usually responsible for the adverse reactions caused by cotrimoxazole, and adverse reactions caused by trimethoprim alone are relatively rare. Nevertheless, cutaneous reactions have been reported and include fixed drug eruption [1-4], linear fixed drug eruption [5], and generalized erythematous skin eruptions [6]. We report a case of enanthema and fixed drug eruption caused by trimethoprim in a patient taking trimethoprim-sulfamethoxazole.

A 46-year-old man with no personal history of allergy presented well-delimited, round-to-oval, erythematous, itchy macules measuring 4 cm in diameter on the dorsum of both hands 2 hours after taking a Septrin tablet (trimethoprim-sulfamethoxazole). The lesions lasted for several days and healed with residual hyperpigmentation. Ten months later, he experienced the same lesions on the hands, upper lip, and oral mucosa after taking an Abactrin tablet (trimethoprim-sulfamethoxazole).

We studied the same patient 20 years later after obtaining his informed consent. Patch testing was performed on involved and uninvolved skin with trimethoprim and sulfamethoxazole at concentrations of 20% and 50% pet [4], with petrolatum as the negative control. No reaction was observed at 48 and 96 hours on previously involved and uninvolved skin of the hands.

As the results of the patch tests were negative and systemic challenge is still the most reliable method of establishing the causal agent in drug reactions, we decided to perform a simple blinded oral challenge with trimethoprim (160 mg) and sulfamethoxazole (800 mg). Six hours after oral challenge with trimethoprim (160 mg) the patient experienced a burning and grazed sensation on the roof of the mouth (Figure). When this lesion resolved, an oral challenge with sulfamethoxazole was performed and its result was negative.

Reports of topical challenge and positive oral challenge with trimethoprim are rare in the literature [1-5]. Because fixed drug eruptions are site-specific, it is essential to perform testing on previously affected sites, although the test results are almost always negative. In the case we report, the patch test results were negative in both involved and uninvolved skin, probably because the patient had experienced the first adverse reaction 20 years before he attended our department. To establish whether trimethoprim was responsible for cutaneous lesions, a challenge test was performed. The reappearance of the lesions in response to the challenge leaves little doubt that the enanthema and fixed eruption were caused by this drug [4]. Involvement of the mucosa receives little attention in the literature [3]; therefore, we think that this case is unusual because enanthema appeared on the roof of the mouth in the oral challenge test (Figure). The oral mucosa was the first site where the lesion developed after oral challenge with trimethoprim, probably because the last dose of trimethoprim was taken 20 years previously; therefore, it seems that it was necessary to take the drug for several days in order to reproduce all the lesions.

In conclusion, we describe a rare case of enanthema and fixed drug eruption due to trimethoprim. Patch test results on involved and uninvolved skin were negative with trimethoprim and sulfamethoxazole, but oral challenge results were positive with trimethoprim. Sulfamethoxazole was not involved in the reaction.

We would like to highlight the importance of testing trimethoprim and sulfamethoxazole independently in order to identify the offending drug in the reaction, and thus to determine the appropriate therapy. There are very few reports in the literature on fixed drug eruption and, in particular, enanthema caused by trimethoprim.

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Dr M Carmen Moreno Escobosa
Allergy Department.
Hospital Torrecárdenas.
Paraje Torrecárdenas, s/n.
04009 Almería, Spain.
E-mail: moreno.escobosa@terra.es

Hypersensitivity Reaction to Darunavir and Desensitization Protocol

MC Marcos Bravo,[1] A Ocampo Hermida,[2] J Martínez Vilela,[3] MT Pérez Rodríguez,[2] MJ Gavilán Montenegro,[1] LJ Arenas Villarroel,[1] C Miralles Alvarez,[2] A Rodríguez Dasilva,[3] C Martínez Vázquez[2]

[1]*Allergology Service, Complejo Hospitalario Universitario de Vigo, Vigo, Spain*

[2]*Internal Medicine Service, Complejo Hospitalario Universitario de Vigo, Vigo, Spain*

[3]*Pharmacy Service, Complejo Hospitalario Universitario de Vigo, Vigo, Spain*

Key words: Antiretroviral agents. Darunavir. Desensitization. Hypersensitivity reaction. Protease inhibitor.

Palabras clave: Antirretrovirales. Darunavir. Desensibilización. Reacción de hipersensibilidad. Inhibidor de proteasa.

Drug hypersensitivity reactions are a serious problem in the management of the HIV-positive patient, and antiretroviral drugs are currently the main cause of these reactions [1]. Amprenavir is the protease inhibitor that most frequently produces adverse cutaneous reactions (up to 28% patients) [2].

Darunavir (Prezista) is a new protease inhibitor and, to our knowledge, no authors have applied desensitization protocols to manage hypersensitivity reaction to this agent [3]. We report a case of cutaneous reaction to darunavir and a desensitization protocol in an HIV-infected patient.

A 17-year-old woman who became infected with HIV when she was 8 years old received zidovudine before beginning highly active antiretroviral therapy (HAART) with a protease inhibitor–based regimen in 1998. In September 2007, she began a regimen containing lopinavir/ritonavir, emtricitabine/tenofovir, and zidovudine. However, in December 2007, her $CD4^+$ T-cell count was 314 cells/μL and her HIV viral load was 4490 copies/mL. In January 2008, she began therapy with enfuvirtide and darunavir. Eight days after starting the new regimen, the patient complained of a pruritic papular erythematous eruption located initially on the ventral surface of the upper extremities, and which later extended to the rest of her body. No systemic symptoms were reported. The patient was treated with oral corticosteroids and antihistamines (fexofenadine 180 mg) and all the antiretroviral drugs were withdrawn. The symptoms disappeared after a few days. The patient had no previous history of atopy or hypersensitivity reactions (including sulfonamide allergy).

A week later, she underwent a single-blind controlled oral challenge with progressively increasing doses of darunavir without ritonavir, in order to rule out its implication in the reaction. The starting dose was 150 mg and, if no symptoms were experienced, the dose was increased 2-fold at 1 hour intervals until the therapeutic dose of 600 mg was reached. Written informed consent was obtained from the patient's mother. Ninety minutes after the last dose (600 mg), the patient presented a pruritic erythematous eruption on her thighs. She was treated immediately with parenteral dexchlorpheniramine and methylprednisolone (60 mg) and received oral treatment with antihistamines and corticosteroids for 3 days.

Because therapy with darunavir had previously been successful, and given the lack of response to previous antiretroviral agents, the patient and her family agreed to try a desensitization protocol.

The patient underwent desensitization in an outpatient regimen (Table) after 22 days of positive oral challenge with darunavir. The drug was administered orally at 30-minute intervals and without pretreatment with antihistamines, corticosteroids, or both. Throughout the desensitization protocol, the patient remained in a specially equipped room where her clinical parameters were monitored. The protocol was started at 25 μg of darunavir (darunavir dissolved in saline solution at a concentration of 20 μg/mL) after an initial dose of 100 mg of ritonavir. The patient reached a cumulative dose of 693 mg in 6.5 hours, with no complications. The following day, she received a dose of 600 mg of darunavir plus 100 mg of ritonavir, with no adverse reactions. At the end of the protocol, darunavir 600 mg bid, ritonavir 100 mg bid, raltegravir 400 mg bid, and tenofovir/emtricitabine 245 mg/200 mg qd were

Table. Desensitization Schedule

Dose Number	Dose Administered	Cumulative Dose, mg
1	25 μg: 1.25 mL (20 μg/mL)	–
2	250 μg: 12.5 mL (20 μg/mL)	–
3	500 μg: 25 mL (20 μg/mL)	–
4	1 mg: 50 mL (20 μg/mL)	1
5	2 mg: 100 mL (20 μg/mL)	3
6	5 mg	8
7	10 mg	18
8	25 mg	43
9	50 mg	93
10	100 mg	193
11	200 mg	393
12	300 mg	693

administered with no complications (8 months of follow-up). Her HIV viral load is <40 copies/mL and her CD4+ T-cell count is 367 cells/μL.

The reaction observed in our patient and confirmed by the positive challenge result is suggestive of a hypersensitivity reaction, although the definitive mechanism remains unclear. This case shows that a desensitization protocol can be a valid approach to HIV patients who experience adverse reactions to darunavir. However, further experience is needed.

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Dr Carmen Marcos Bravo
Servicio Alergología
Complejo Hospitalario Universitario de Vigo
C/ Pizarro, 22
36204 Vigo (Pontevedra), Spain
carmen.marcos.bravo@sergas.es

Multicenter Investigation to Assess the Prevalence of Ambrosia Pollen Allergy inTuscany

S Testi,[1] A Carabelli,[2] L Cecchi,[3] C Giacomelli,[4] G Iannello,[5] V Rocchi,[6] O Rossi,[7] I Spadolini,[8] F Vannucci,[9] P Campi[1]
[1]*Allergy and Clinical Immunology Unit, Azienda Sanitaria di Firenze, Florence, Italy*
[2]*Section of Allergy, Cisanello Hospital, Pisa, Italy*
[3]*Allergy Clinic, Azienda Sanitaria di Firenze, Florence, Italy*
[4]*Section of Allergy, Versilia Hospital, Lucca, Italy*
[5]*Allergy Clinic, Pontedera Hospital, Pisa, Italy*
[6]*Clinical Immunology Unit, Department of Internal Medicine, University of Pisa, Pisa, Italy*
[7]*Section of Immunoallergology and Respiratory Diseases, University of Florence, Florence, Italy*
[8]*Anallergo Laboratories, Florence, Italy*
[9]*Allergy Clinic, Pistoia Hospital, Pistoia, Italy*

Key words: Allergy. Ragweed pollen. Epidemiology. Tuscany.

Palabras clave: Alergia. Polen de ambrosia. Epidemiología. Toscana.

Ragweed was first found in Italy in the regions of Piemonte and Liguria in 1902. The most affected regions of Italy today are Lombardia and Friuli Venezia-Giulia, in the northeast [1-3]. To our knowledge, no published data exist on the distribution of ragweed plants in the central or southern parts of Italy. In recent years, there has been an increase in the pollen count of ragweed in Tuscany, although no plants have been found to date. The pollen count has been reported to be above the clinical threshold several times in both Florence and Pistoia [4].

Skin prick tests were carried out on consecutive patients in 8 allergy centers in northern Tuscany (from Florence to the Tyrrhenian coast) with the following aeroallergens (Anallergo, Florence, Italy): *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*; cat and dog dander; grasses (Graminaceae mix, *Artemisia vulgaris*, *Parietaria judaica*); trees (*Cupressus arizonica, Corylus avellana, Quercus ilex*, *Olea europaea*); and fungi (*Alternaria tenuis*). Latex and *Ambrosia elatior* were also added.

Histamine at 10 mg/mL and glycerol phosphate buffer 50% were used as a positive and negative control, respectively. The skin reaction measured 15-20 minutes after the test was considered positive if the diameter of the wheal was ≥4 mm compared to the negative control and was accompanied by erythema [5].

A total of 845 adults patients were tested (508 females and 337 males) and 111 reacted to Ambrosia pollen (13.14%). Of these, 40 reported respiratory symptoms in the summer-autumn period (36%). Among ragweed-positive patients, 22 were monosensitized (2.6%), 9 with symptoms in summer-autumn (1%). It should be noted that 77 out of 111 (69.3%) patients were also sensitized to mugwort, confirming the well-known cross-reactivity between both pollen extracts. This result could lead to an overestimation of the sensitization rate.

Despite the absence of this herbaceous species in the region, Ambrosia pollen is transported in air masses from Eastern Europe [1]. We therefore assessed the percentage of individuals sensitized to this pollen, and found that in Tuscany the figure was far from negligible and may even have been even higher if the study had taken into account only patients with respiratory symptoms. To our knowledge, this is the first specific study to examine sensitization to a pollen that is only occasionally detected.

Curiously, the percentage of sensitized individuals decreases the closer the distance to the Tyrrhenian coast, where it is 0%. Since ragweed pollen presumably comes from Eastern Europe, one could speculate that the pollen count decreases westward. Sensitization to Ambrosia warrants further study, perhaps involving a wider area.

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Sergio Testi
Allergologia ed Immunologia Clinica
Azienda Sanitaria di Firenze
Nuovo Ospedale San Giovanni di Dio
Via di Torregalli 3
50143 Firenze, Italy
E-mail: sergio.testi@asf.toscana.it

