

Association Between Pollen Exposure and Nasal Cytokines in Grass Pollen–Allergic Children

Di Cara G¹, Panfilì E¹, Marseglia GL², Pacitto A¹, Salvatori C¹, Testa I¹, Fabiano C³, Verrotti A³, Latini A¹

¹Università degli Studi di Perugia, Dipartimento di Scienze Chirurgiche e Biomediche, Perugia, Italy

²Unità di Pediatria Generale e Specialistica, Dipartimento di Scienze Clinico-Chirurgiche Diagnostiche e Pediatriche, Fondazione IRCCS Policlinico San Matteo di Pavia, Pavia, Italy

³U.O.S.D. Pediatria D.U. e Pronto Soccorso Pediatrico, Dipartimento Materno Infantile, Università degli Studi dell'Aquila, L'Aquila, Italy

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Allergic rhinitis (AR) is the most common chronic disorder in the pediatric population, with up to 40% of children affected [1]. AR and its comorbidities have a profound impact on the daily lives of children, yet the disease remains underdiagnosed and undertreated in this group [2].

The Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines classify rhinitis into AR and nonallergic rhinitis based on the presence and absence of allergic sensitization. AR arises due to IgE-mediated mechanisms and is usually characterized by a typical cellular inflammatory pattern in the nasal mucosa involving eosinophils and mast cells.

A recent study on nasal cytology demonstrated a close relationship between increased counts of eosinophils and mast cells and pollen exposure in *Parietaria*-sensitized adults, with cell counts better reflecting inflammation, and thus clinical symptoms, than the pollen count itself [3].

However, nasal cytology is used less commonly in children than in adults, often because of poor cooperation by the pediatric patient. Moreover, the increase in nasal inflammatory cells is more influenced by other confounding factors such as infectious agents.

Nasal mediators in nasal lavage fluid have been evaluated in clinical practice to validate allergen-specific nasal challenges, in which levels of eosinophil cationic protein (ECP) and tryptase are used as markers of immediate and delayed inflammation. Both correlate well with allergen exposure [4,5].

The role of these mediators in clinical practice has yet to be completely defined, although the identification of new

types of rhinitis, such as local AR, highlights their potential importance in both diagnosis and follow-up [6].

We studied children sensitized to grass pollens to compare the relationship between pollen exposure and eosinophils and mast cell cytokines (ie, ECP and tryptase), which was evaluated using a nasal incubation method to verify the potential of these markers for monitoring nasal inflammation in a pediatric population.

The study population comprised 38 children (23 boys, 15 girls; mean age, 11.5 years) diagnosed with AR according to the ARIA guidelines, a positive skin prick test result for grass pollen, with negative results for the other common aeroallergens, and clinical symptoms consistent with sensitization during the previous pollen season. Skin prick test results were considered positive if the wheal diameter exceeded 5 mm. The exclusion criteria were a previous history of specific immunotherapy (subcutaneous or sublingual), a diagnosis of nonallergic rhinitis, presence of other diseases (chronic, immunologic, and hematologic-oncologic), and sensitization to other allergens.

Nasal cytokine sampling: Nasal tryptase and ECP sampling was performed using ELISA (UniCAP Tryptase System FEIA and UniCAP ECP System FEIA, Pharmacia) adapted for mucosal sampling based on a method described elsewhere [5-6].

All children were sampled every 2 weeks before the beginning and after the end of the pollen season (March 1, 2014 to September 30, 2014). The nasal tests were accompanied by recording of the clinical symptom score.

Grass pollen count: We recorded 22 mean weekly grass pollen concentration values and peaks (grains/m³ of air) in Perugia, Italy between March 1, 2014 and September 30, 2014. The pollen season started on April 23 and ended on June 27, with 2 pollen peaks in the second and third weeks of May and in the first 2 weeks of June. In 2014, an unusual peak was also observed in the pre-autumn period, with a lower pollen peak from the fourth week of August to the second week of September.

Evaluation of nasal symptoms: Clinical symptoms were evaluated using the Rhinoconjunctivitis Total Symptom Score (RTSS). Each patient scored 6 individual rhinitis symptoms on a 4-point scale. Scores ranged from 0 to 18, with higher scores indicating more severe allergy symptoms.

Nasal ECP levels began to increase at the beginning of April and persisted until the end of June, with mean values always present during exposure; a second increase in ECP values was observed between late August and September (Figure, A). Nasal tryptase levels were more variable, better reflecting the pattern of pollen concentration in each of the months studied, with higher peaks during the 2 pollen peaks (Figure, B).

RTSS values were higher during both pollen peaks (7.12 [2.14] and 7.84 [3.27]). A smaller increase in the RTSS

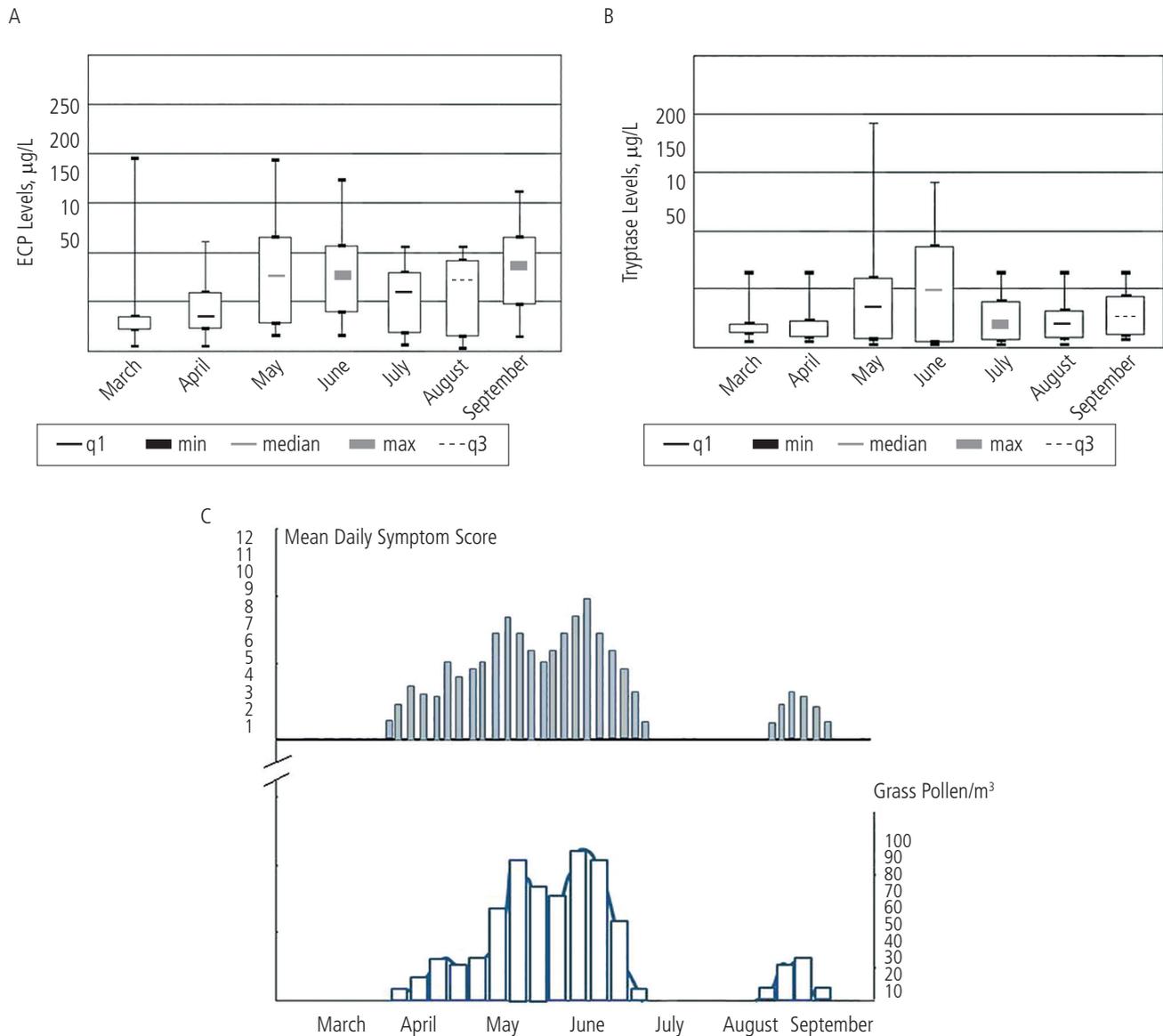


Figure. A, Eosinophil cationic protein variations during grass pollen exposure. B, Tryptase variations during grass pollen exposure. C, Rhinoconjunctivitis Total Symptom Score and pollen count.

was also observed in most patients between the end of August and the first half of September (4.23 [2.15]) (Figure, C).

We observed a clear relationship between nasal cytokine levels, grass pollen exposure, and severity of symptoms, with an increase in nasal ECP levels throughout the pollen season and greater variability in nasal tryptase levels, which were apparently more related to air concentrations and nearly mirrored pollen peaks. This different pattern of response suggests a more specific role of tryptase as a marker of allergen exposure, since it better reflects the immediate phase of reaction to the allergen. Variations in ECP were less specific and had higher values, even during periods of reduced exposure to pollen, possibly owing to the persisting late-phase activation of allergic inflammation throughout the pollen season. Both cytokines disappeared a few days after the end of the pollen season, thus reflecting the end of the inflammatory stimulus.

Our data suggest that evaluation of nasal cytokines is useful when attempting to establish a correlation between exposure and sensitizing pollens and symptoms in a pediatric population. This approach could also prove useful in polysensitized patients, a relevant group of allergic children, to better define which allergens are most responsible for clinical symptoms.

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Conflicts of Interest

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Giuseppe Di Cara

Università degli Studi di Perugia
Dipartimento di Scienze Chirurgiche e Biomediche
Piazza Lucio Severi 1
06123 Perugia - Italy
E-mail: giu.dicara@gmail.com

Identification of a Lipid Transfer Protein as a New Allergen From *Morus alba* Pollen

El-Qutob D¹, Bartolome B², Cuesta J³, Mir A^{4*}, Alberola A^{5*}, Pastor-Vargas C⁶

¹Unit of Allergy, University Hospital of La Plana, Vila-Real, Spain

²R&D Department, Roxall, Bilbao, Spain

³Allergy Service, Instituto de Investigaciones Sanitarias Fundación Jiménez Díaz, UAM, Madrid, Spain

⁴Department of Medicine of the Faculty of Medicine of the University of Valencia, Valencia, Spain

⁵Department of Physiology of the Faculty of Medicine of the University of Valencia, Valencia, Spain

⁶Department of Immunology, Instituto de Investigaciones Sanitarias Fundación Jiménez Díaz, UAM, Madrid, Spain

*Both authors contributed equally to this manuscript.

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Palabras clave: Polen de *Morus alba*. Proteína transferidora de lípidos no-específica. Profilina. Polisensibilización.

Pollen allergy has a significant clinical impact in Europe [1], and the prevalence of allergic rhinitis induced by pollen has been increasing in recent years [2].

Mulberry (*Morus alba*) pollen is one of the most allergenic pollens within the Moraceae family in Spain [3], and cross-reactivity between allergens from different species of the genus *Morus*, and even between the members of the Moraceae family, including paper mulberry (*Broussonetia papyrifera*), breadfruit (*Artocarpus altilis*), *Ficus* species (eg, *Ficus elastica*), and jackfruit (*Artocarpus heterophyllus*) [4], is well known.

Despite the presence of *M alba* pollen in the air and sensitized patients presenting positive results in skin prick tests (SPT) and/or determinations of serum specific IgE to mulberry pollen extract, the clinical relevance of sensitivity to the allergen is unknown. To date, 3 allergens have been identified in fruits from the Moraceae family, namely, Mor a 1 (member of the Bet v 1-like family), Mor a 4 (profilin) from white mulberry (*M alba* fruit), and Mor n 3 (nonspecific lipid transfer protein [nsLTP]) from black mulberry (*Morus nigra*). All 3 belong to different panallergen groups.

The aim of this study was to evaluate the allergenic components of mulberry pollen extract and thus identify new allergens.

The study population comprised 45 patients sensitized to *M alba* pollen (30 women and 15 men; mean [SD] age, 33.72 [13.42] years) who met the following criteria: (1) seasonal rhinitis and/or bronchial asthma from February to April; (2) age >18 years; (3) a positive SPT result to *M alba* pollen and/or specific IgE values >0.35 kU_A/L to this pollen measured by the ImmunoCAP System (Thermo Fisher

Scientific); (4) a positive result in a conjunctival provocation test with different concentrations of *M alba* pollen extract; and (5) no previous treatment with specific immunotherapy. The protocol was approved by the Ethics Committee of the University of Valencia, Valencia, Spain, and patients included in the study provided their informed consent.

M alba pollen was delipidized before being extracted by magnetic stirring under shaking in 50 mM phosphate-buffered saline (pH 7.5) for 16 hours at 4°C. After centrifugation, the supernatant was dialyzed against distilled water. The dialyzed extract was filtered through a 0.22- μ m-pore diameter membrane and freeze-dried. The extract was diluted in glycerinated phenol saline solution at a concentration of 5 mg/mL, and SPTs were performed following European guidelines [5].

SDS-PAGE was performed with 12.5% acrylamide following Laemmli [6] and with 16.5% acrylamide and tris-tricine buffer as described by Schägger and von Jagow [7]. Immunoblotting and identification of proteins by tandem mass spectrometry were performed as previously described [8].

SDS-PAGE (16.5% tricine) with *M alba* pollen extract after Coomassie Blue staining revealed 3 small proteins with molecular masses of 10 kDa, 8.5 kDa, and 7 kDa (Figure). SDS-PAGE immunoblotting (16.5% tricine) with *M alba* pollen extract and patients' sera revealed a double IgE-reactive band of 14-15 kDa in 21.8% of the sera and 3 low-molecular-weight bands (<14 kDa) in 87.5% of the sera. Other bands with lower frequencies were also observed (Figure). An immunoblotting-inhibition assay (12.5% acrylamide) was carried out with patient sera using *M alba* pollen extract in the solid phase and various purified pollen profilins as inhibitors: total inhibition of IgE binding on the 14-15-kDa double band was observed for all pollen profilins. These results indicated that this double band was the profilin from *M alba* pollen (results not shown). Mass spectrometry revealed 3 IgE-binding bands smaller than 14 kDa: an nsLTP (10 kDa), a ubiquitin-

like protein (8 kDa), and a cystatin-like protein (7 kDa). An immunoblotting-inhibition assay (12.5% acrylamide) with *M alba* pollen extract in the solid phase and using a patient's serum (the same serum used in Figure, lane 8) was carried out with pollen extracts (grass and olive), Pru p 3 (nsLTP from peach), and Par j 1 + Par j 2 (nsLTP from *Parietaria judaica* pollen) as inhibitors. Total inhibition of IgE binding on the 14-15-kDa-double band (profilin) and on the nsLTP-10 protein was observed for both pollens, whereas the Par j 1 + Par j 2 sample only inhibited IgE-binding to the nsLTP-10 protein. No inhibition was observed when the Pru p 3 sample was used as an inhibitor (Figure). An immunoblotting-inhibition assay (12.5% acrylamide) showed that pollens from *P judaica* and *Olea europaea* were also able to inhibit IgE binding to the ubiquitin-like protein and the cystatin-like protein (results not shown).

No allergens have been identified from *M alba* pollen extract to date, although its fruit has yielded 2 proteins, namely, Mor a 1 (Bet v 1-like family) and Mor a 4 (profilin). We successfully identified the *M alba* pollen extract profilin as a double band of 14-15 kDa that appeared in 21.8% of patients' sera. Two previous studies detected 2 unidentified IgE-reactive proteins of 9 kDa [9] and 10 kDa [10] in *M alba* pollen extract. In our study, the identification of protein bands by mass spectrometry recognized 3 new allergens <14 kDa: a 10-kDa LTP (nsLTP-10), an 8-kDa ubiquitin-like protein, and a 7-kDa cystatin-like protein. Furthermore, immunoblotting-inhibition analysis revealed the existence of cross-reactivity between nsLTP-10 and Par j 1+Par j 2, whereas no cross-reactivity was detected between nsLTP-10 and Pru p 3. The proteins that cross-reacted with the ubiquitin-like protein and the cystatin-like protein are most likely present in pollens from *P judaica* and *O europaea*, as can be deduced from the immunoblotting-inhibition results.

M alba pollen is an allergenic source with several IgE binding components. We confirmed the significance of profilin

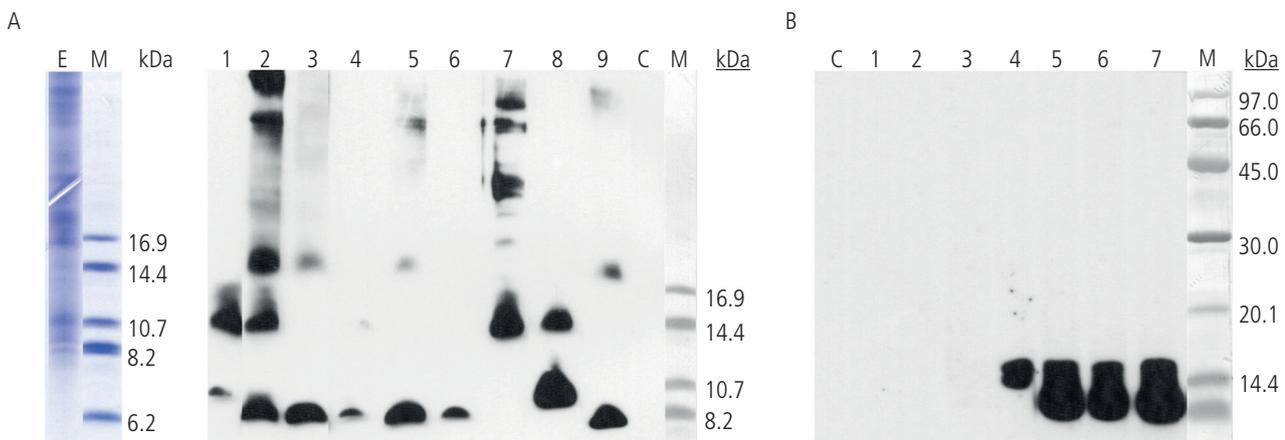


Figure. A, SDS-PAGE Coomassie Blue staining and immunoblotting (16.5% acrylamide-tricine buffer) with patients' sera. Lane E, extract from *M alba* pollen; lanes 1-9, patients' sera; lane C, control serum (pool of sera from nonatopic individual); lane M, molecular mass standard. B, Immunoblotting-inhibition (12.5% acrylamide) with *M alba* pollen in the solid phase and using the same serum assayed in lane 8 (Panel A). Lanes 1-7, patient serum previously incubated with *Morus alba* pollen extract (lane 1), with *Parietaria judaica* pollen extract (lane 2), with *Olea europaea* pollen extract (lane 3), with Par j 1+Par j 2 (lane 4), with Pru p 3 (lane 5), with lamb extract (lane 6), and with bovine serum albumin (lane 7).

sensitization in patients allergic to *M alba* pollen extract and cross-reactivity with other pollen profilins from unrelated plant families. Further studies are needed to determine the amino acid sequence of these allergens.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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David El-Qtob

Unit of Allergy of University Hospital of La Plana, Vila-Real
Carretera Vila-Real-Burriana Km. 0.5
12540 Vila-Real (Castellon), Spain
E-mail: elqtob@comv.es

Eosinophilic Esophagitis After Egg Oral Immunotherapy in an Adult With Egg-Allergy and Egg-Bird Syndrome

García Rodríguez R, Méndez Díaz Y, Moreno Lozano L, Extremera Ortega A, Gómez Torrijos E
Hospital General Universitario de Ciudad Real, Ciudad Real, Spain

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Palabras clave: Esófagitis eosinofílica. Inmunoterapia oral. Síndrome ave-huevo. Adulto. Disfagia. Respuesta dual.

Egg allergy is much less common in adults than in small children. In adults, it is mostly caused by allergens in the yolk as a result of sensitization to inhaled bird allergens and is known as bird-egg syndrome [1]. In recent years, the number of reports on oral immunotherapy (OIT) in food-allergic children has been increasing [2], although no adult cases have been published. Eosinophilic esophagitis (EoE) has been diagnosed in children treated with OIT [3-4].

A 55-year-old woman was diagnosed with allergy to egg white when she was 1 year old. Since childhood, she had been exposed to chickens on a poultry farm. Despite following an egg-free diet, she presented with anaphylaxis upon eating trace amounts of egg as a hidden allergen. During adolescence, she began to experience epigastric pain when she ate any undercooked poultry meat. When she was 25 years old and

pregnant, she began to experience episodes of bronchospasm that worsened on direct exposure to poultry. She has since avoided exposure to birds and remains free of respiratory symptoms.

The results of skin prick tests to commercial egg white were positive (5 mm), egg-yolk (3 mm), ovalbumin (6 mm), ovomucoid (7.5 mm), and chicken meat extracts (3.5 mm) (ALK Abelló). Prick-by-prick tests were also positive with pasteurized egg white (9.5 mm) and raw egg yolk (8.5 mm). Specific IgE to yolk was 4.2 kU_A/L (ImmunoCAP, Thermo Fisher), egg white 11.3 kU_A/L, ovalbumin 3.3 kU_A/L, and ovomucoid 0.62 kU_A/L.

SDS-PAGE immunoblotting (Figure) of egg yolk, egg white, and feathers with commercial nonglycerinated extracts (1/20 wt/vol) (ALK Abelló) was performed with the patient's serum [5]. This technique revealed a single band of approximately 70 kDa that bound IgE in chicken feathers and was also present in the yolk extract. This corresponded to Gal d 5 or α -livetin. A 31- to 37-kDa band was detected in yolk and may correspond to Gal d 6. Bands of 28 kDa, 45 kDa, and 75 kDa corresponding, respectively, to Gal d 1 (ovomucoid), Gal d 2 (ovalbumin), and Gal d 3 (ovotransferrin) were also detected in egg white. A band of approximately 20 kDa was present in egg yolk. Its significance is uncertain, although it may correspond to vitellogenin II, a low-molecular-weight protein in egg yolk.

Single blind oral challenge tests were positive to boiled egg white and raw egg yolk.

The patient subsequently underwent rush desensitization protocols with raw egg yolk and raw egg white administered separately [6]. The raw egg yolk doses elicited digestive symptoms, although the patient could tolerate half a raw egg yolk after 5 days. Desensitization to raw egg white failed owing to frequent systemic reactions, and desensitization was subsequently undertaken with whole cooked egg (omelette); the patient finally achieved tolerance to half a cooked egg in 6 days. During the maintenance phase, she was instructed to eat half a cooked egg, although the dose had to be reduced 2 months later because of significant epigastric and abdominal pain after each dose. Since then, she has only eaten baked egg in pastries or cookies.

Three years later she complained of frequent episodes of dysphagia, knots in her neck, and heartburn upon eating any food. Even though the patient ate only baked egg and well-cooked poultry meat, an esophago-gastro-duodenoscopy with 8 biopsies (4 from the distal third and 4 from the mid-upper esophagus) revealed 15 eosinophils/high-power field (eos/HPF) in the mid-upper esophagus biopsy specimen; therefore, the patient was diagnosed with EoE.

After 2 months of therapy with omeprazole (40 mg bid), a new biopsy of the esophageal mucosa revealed 0 eos/HPF. She later followed a diet excluding egg and poultry for 3 months without omeprazole, and only 4 eos/HPF were detected. The patient remains asymptomatic after 1 year following the avoidance diet.

The case we report was characterized by several episodes of anaphylaxis owing to class 1 allergy to egg white that first appeared during childhood. The patient subsequently experienced digestive symptoms upon eating undercooked

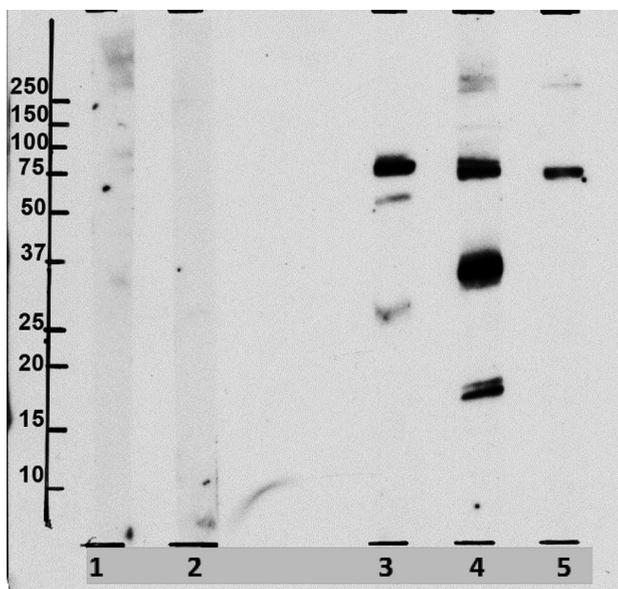


Figure. Results of SDS-PAGE immunoblotting.

poultry and asthma after being exposed to birds (class 2 egg allergy). The patient was also sensitized to Gal d 6, which is exclusive to egg yolk. Therefore, primary sensitization to yolk was through the digestive tract. The patient was cosensitized to egg white and egg yolk allergens and cross-sensitized to egg via the inhalation of bird allergens. In their recent molecular and immunological analysis, De Silva et al [7] revealed that cosensitization to yolk allergens affected 36% of egg white-allergic patients [7]. More studies are needed to confirm the clinical implications of this finding, that is, the importance of egg yolk in egg-allergic children.

Follow-up was similar to that of another case report of a teenager desensitized to milk [4] who developed EoE, with poor tolerance of the food doses in the maintenance phase and symptoms of esophageal dysfunction some months or years after the OIT induction phase. EoE is much more frequent after milk OIT and has seldom been described after desensitization with egg [8].

Until recently, proton pump inhibitors and exclusion diets were considered mutually exclusive [9]. Lucendo et al [10] reported on 7 patients who responded to both. We also observed a dual response, since the patient experienced clinical and histological remission, not only with high-dose omeprazole therapy, but also with the withdrawal of egg and poultry.

In conclusion, we report the first case of a patient with egg allergy and egg-bird syndrome who underwent OIT with raw egg yolk and, subsequently, with whole egg. The patient was later diagnosed with EoE, which remitted with high doses of omeprazole and an egg-free diet. At least partial tolerance can be achieved with OIT to egg in adult patients with this diagnosis. Patients undergoing OIT should be monitored in the long term to detect the possible onset of symptoms of esophageal dysfunction. Once the diagnosis of EoE has been established, both therapeutic options should be offered.

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Conflicts of Interest

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Elisa Gomez Torrijos

Hospital General Universitario de Ciudad Real
Sección de Alergología
13005 Ciudad Real, Spain
E-mail: egomez.torrijos@gmail.com

Physician, Beware! Drug Fever Without Skin Rashes Can Be Caused by Minocycline

Gu W¹, Shi D¹, Mi N², Pang X¹, Liu W¹

¹Department of Dermatology, General Hospital of Air Force, Beijing, China

²Hospital of Beijing Technology and Business University, Beijing, China

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Key words: Acne. Adverse effect. Fever. Minocycline. Treatment.

Palabras clave: Acné. Efectos adversos. Fiebre. Minociclina. Tratamiento.

Minocycline is widely prescribed for the treatment of skin and venereal disease. Although a relatively safe drug, its most common adverse effects are nausea, vomiting, dizziness, and cutaneous symptoms. Severe adverse effects of minocycline include hepatitis, systemic lupus erythematosus, and hypersensitivity syndrome [1,2]. Here, we present a case of severe adverse effects, including high fever due to oral minocycline.

A 24-year-old woman was taking minocycline at her usual dose (100 mg/d) for treatment of acne. After 6 days, she went to hospital with fever (39.5°C), dizziness, chest tightness, and eye pain. She was diagnosed with a cold and treated. Minocycline was discontinued. Her fever and malaise lasted for 5 days. Three days later, she restarted minocycline as treatment for acne. High fever (39.2°C) and the symptoms reported above recurred within a few hours. Given the link between fever and minocycline intake, she was referred to our department. Her blood pressure, respiratory rate, and heart rate were normal. Physical examination showed neither swelling of the throat nor enlarged lymph nodes. A more detailed physical examination revealed no rash on any part of her body. Magnetic resonance imaging of the brain and computed tomography of the chest revealed no abnormalities. Additional laboratory findings disclosed eosinophilia (white blood cell count, $7.5 \times 10^9/L$; eosinophils, $1.2 \times 10^9/L$), normal C-reactive protein level, and normal liver and kidney function. Blood culture for bacteria and testing for antihuman herpesvirus 6 IgG were negative. Based on her history of therapy with minocycline and typical clinical presentation, she was diagnosed as having minocycline-induced drug fever. The fever and other symptoms improved significantly after discontinuation of minocycline and oral prednisolone (30 mg/d). Her clinical course is illustrated in the Figure. Application of the Spanish Pharmacovigilance System probability algorithm (modified Karch-Lasagna algorithm) revealed the causality between the adverse drug reaction and the drug to be definite [3,4].

Minocycline-induced fever is rare but can be serious. Grim et al [5] reported a case of severe drug fever complicated with malaise that was overlooked during long-term therapy with minocycline [5]. In the case we report, the patient was initially

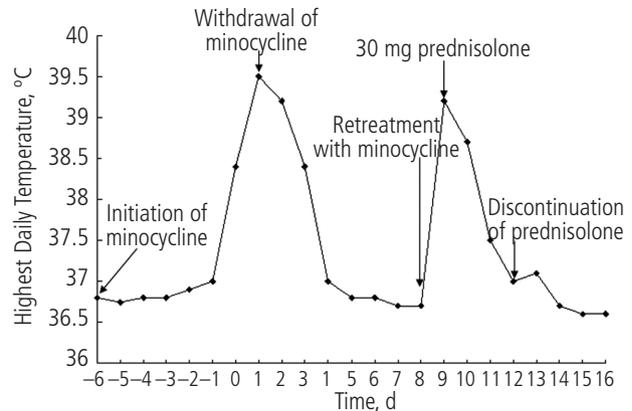


Figure. Highest daily temperature during the clinical course. The fever deteriorated during therapy with minocycline and improved when the drug was discontinued.

diagnosed as having a cold. Discontinuation of minocycline led to improvement of symptoms. Reinitiation of treatment—which acted as an oral challenge test—induced fever again and thus confirmed the diagnosis. The adverse effects of minocycline vary from patient to patient and include dizziness, nausea, and skin rashes such as urticaria [6]. The patient presented fever complicated with dizziness, chest tightness, and eye pain. However, in contrast with previous cases, she did not present skin rash. The diagnosis of drug fever is often difficult, and a careful review of the clinical presentation and medication history can confirm the diagnosis. Although this patient had severe symptoms, the diagnostic criteria for drug reaction with eosinophilia and systemic symptoms were not fulfilled because of the absence of skin rash and other systemic features [7,8].

As well as illustrating a rare adverse reaction to minocycline, our report emphasizes the need for physicians and dermatologists to be on the alert for the unusual drug-induced fever, especially in patients without apparent skin rashes. Notification of adverse drug reactions is mandatory for all healthcare professionals and pharmaceutical companies in many countries. For example, the Spanish Pharmacovigilance Service (RDL 1/2015, 24 July) established a system to monitor the safety of authorized medicinal products and detect any change to their risk-benefit balance [9].

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Conflicts of Interest

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Wei Liu

No. 30 Fucheng Road
Haidian District
Beijing 100142, China

Donkey's Milk Is Well Accepted and Tolerated by Infants With Cow's Milk Food Protein-Induced Enterocolitis Syndrome: A Preliminary Study

Mori F¹, Sarti L¹, Barni S¹, Pucci N¹, Belli F², Stagi S³, Novembre E¹
¹Allergy Unit, Department of Pediatrics, Anna Meyer Children's University Hospital, Florence, Italy
²Nutrition Unit, Department of Pediatrics, Anna Meyer Children's University Hospital, Florence, Italy
³Department of Health Sciences, University of Florence, Anna Meyer Children's University Hospital, Florence, Italy

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Palabras clave: Niños. Leche de vaca. Leche de burra. Síndrome de enterocolitis inducida por proteína alimentaria.

Food protein-induced enterocolitis syndrome (FPIES) is a type of non-IgE-mediated food hypersensitivity reaction that is characterized by vomiting, diarrhea, lethargy, and, in some cases, hypovolemic shock and metabolic acidosis. The diagnosis is based on the clinical history and food challenge when appropriate [1]. Cow's milk proteins (CM) and soy milk (SM) proteins are among the most common triggers of FPIES. Negative skin test and cow's milk serum specific IgE results make it possible to differentiate between FPIES and typical CM allergy.

When treating an infant with CM FPIES in the first 6-12 months of life, physicians should suggest a safe and nutritionally appropriate alternative to parents.

Donkey's milk (DM) is available in many regions of Italy. We tested tolerance to DM in 6 children with CM FPIES who were referred to our allergy unit over a 2-year period (2015-2016).

Prick-by-prick testing with CM, SM, casein-based extensively hydrolyzed formula (eHF), DM, and goat's milk was performed according to the recommendations of the European Academy of Allergy and Clinical Immunology. All 6 patients were diagnosed with CM FPIES within 6 months of age according to the criteria of Miceli Sopo et al [1]. An oral provocation test (OPT) was performed according to the protocol of Leonard and Nowak-Wegrzyn [2] in a hospital setting with emergency facilities available.

The final dose of DM and/or SM was 150-200 mL of the whole food, because DM contains 3.3 g of protein in 200 mL.

Tolerance of SM or DM was checked within 3 months of the diagnosis of CM FPIES. DM was provided free to the infants tested, as it is very expensive in some regions of Italy.

The nutritional state of all 6 children was evaluated before feeding with DM. We recorded weight, height, and blood levels of calcium, vitamin D, and insulin-like growth factor (IGF1).

The demographic and allergy profiles of the 6 infants are summarized in the Table. Three of the 6 children had a positive

Table. Demographic Characteristic and Allergy Profiles

No.	Sex	CM FPIES	Reactions	Age at Diagnosis, mo	Allergy tests (P + P, mm)					Serum specific IgE, kU _A /L	CM OPT	Soy OPT	eHF OPT	Donkey OPT
					eHF	CM	GM	SM	DM					
1	F	1.5	Repetitive vomiting after 60 minutes	3	0	0	0	0	0	NP	Positive (vomiting, pallor, hypotonia after 60 minutes; intravenous ondansetron and fluids)	T	NA	T
		2	Repetitive vomiting after 60 minutes											
2	F	2	Repetitive vomiting and lethargy (after 1.5-2 hours)	3	0	0	0	0	0	NP	NP	T	T	T
		2.5	Repetitive vomiting and lethargy (after 1.5-2 hours)											
		3	Repetitive vomiting and lethargy (after 1.5-2 hours)											
3	F	3	5 episodes of repetitive vomiting after 90 minutes	3	0	5	0	0	0	10	NP	T	T	T
4	F	6	Repetitive vomiting and lethargy after 90 minutes	6	0	0	0	0	0	NP	ED (intravenous ondansetron and fluids)	T	NT	T
5	F	4	Repetitive vomiting and lethargy after 2 hours	5	0	0	0	0	0	0	Positive (vomiting after 120 minutes)	NT	NA	T
6	M	2.5	Repetitive vomiting and lethargy after 90-120 minutes	6	NT	0	0	0	0	NP	NP	NT	NA	T
		4.5	Repetitive vomiting and lethargy after 3 hours											

Abbreviations: CM, cow's milk; DM, donkey's milk; ED, emergency department; eHF, extensively hydrolyzed formula; F, female; GM, goat's milk; M, male; NA, not accepted; NP, not performed; NT, not tested; OPT, oral provocation test; P+P, prick by prick; SM, soy milk; T, tolerated.

family history of atopy, but none of the infants had atopic dermatitis. Two out of the 6 children were diagnosed by oral provocation test with CM proteins, and 1 of the 6 children was diagnosed during an acute FPIES episode. The diagnosis was confirmed based on the clinical history (ie, ≥ 2 typical episodes) in 3 of the 6 children [1]. One of the 6 children had CM-specific IgE (atypical FPIES). However, even when that child was excluded, all of the remaining 5 children with CM-induced enterocolitis syndrome tolerated DM.

Most of the children also tolerated SM (4 out of 6), and eHF was a safe but unpalatable alternative (3 out of 6 children

would not consume the eHF because of its taste). The mothers of the first 3 children (Table), who were diagnosed as having CM FPIES at 3 months of age, were advised to breastfeed their babies until 5 to 6 months of age. All 6 children reached the 6-month follow-up period (from 6 months of age until 12 months), maintained the same weight and height percentiles, and had no deficit in calcium, vitamin D, or IGF1 (data not shown).

We found that DM was a safe and well-tolerated alternative in 6 children with CM FPIES. Moreover, it is nutritionally adequate in weaned infants. As for SM, it has been reported that 65% [3], 43.5% [4], and 37% [5] of children with CM FPIES

also reacted to SM. The authors consequently encouraged breastfeeding and recommended using casein-based eHF or an amino acid formula. Reactions to SM should be assessed in a hospital setting.

Our findings differ from those of Katz et al [6], who reported that of 44 Israeli children with FPIES triggered by CM, none reacted to other foods, including SM. Mehr et al [7] reported that of 35 Australian children, none reacted to either SM or CM.

In a multicenter trial conducted by Sopo et al [8], the authors showed that, once again, none of the children reacted to CM and SM. The authors of the 3 previously mentioned studies [6-8] recommended SM as a valid alternative in children with CM FPIES. Moreover, SM-based formulas have moderate palatability and provide appropriate nutrition. The present study also showed that 4 out of 5 patients with CM FPIES tolerated SM. According to our results and taking into account that DM does not have any nutritional advantage over SM, we could only assume that DM is useful as an additional alternative in children with CM FPIES who do not tolerate SM or eHF.

SM-based formulas are contraindicated for the treatment of infants with some forms of non-IgE-associated gastrointestinal CM hypersensitivity. In such cases, DM should be recommended. DM is also favored because of its pleasant taste and the positive feedback from patients who have tried it.

Consensus guidelines from the World Allergy Organization recommend the use of eHF, which has good nutritional value but is expensive and unpalatable.

The literature contains few studies on other mammalian milk products. Thus far, cross-reactivity between foods has not yet been described in FPIES.

Shek et al [9] reported casein to be the major allergen in FPIES. Consequently, goat's milk or sheep's milk should be avoided because of the high degree of homology with CM and its poor nutritional properties [9].

Among mammalian milk products, DM shows little cross-reactivity with CM.

The nutritional composition of DM is similar to that of human milk. Its high lactose content not only makes DM palatable, but also stimulates intestinal absorption of calcium. Moreover, Monti et al [10] found that DM contains levels of casein and whey proteins similar to those in human milk, as well as a high concentration of essential amino acids. However, the low fat content of DM means that it has a low energetic value [10], thus limiting its use for infants on an exclusively milk-based diet. The authors found that DM was tolerated by 13 children with non-IgE-mediated gastrointestinal symptoms.

This is the first report to show that DM is well accepted and tolerated by children diagnosed with CM FPIES. However, our sample (6 infants) was too small to enable us to recommend DM as a substitute for CM. Despite major concerns regarding the nutritional properties of DM in the first 6 months of life, our results indicate that it could be considered a valid alternative in weaned infants (older than 5-6 months). Well-designed clinical studies are needed to confirm our findings.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Simona Barni

Viale Pieraccini 24
50134 Florence
Italy

E-mail: simonabarni@hotmail.com

An Unusual Case of Contact Dermatitis Caused by a Pacemaker Implanted for Neurostimulation

Pérez González EL, Medina Alfaro I, Iglesias Cadarso A, Boteanu C
Allergy Section, Hospital Universitario Puerta de Hierro,
Majadahonda, Spain

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Key words: Contact Dermatitis. Silicone allergy. Pacemaker allergy.

Palabras clave: Dermatitis de contacto. Alergia a la silicona.

Neurostimulation with pacemakers can be used to treat pain. The pacemaker generates electrical impulses that are controlled by means of an electrode signal, thus blocking nerve pain. The generator and the electrodes are coated with silicone, which exhibits negligible allergenicity, in order to prevent adverse reactions to the other materials used in the pacemaker.

We report a case of hypersensitivity to silicone and rhodium in a patient with a pacemaker implanted for neurostimulation.

A 42-year-old man was referred to our allergy department with pain at the insertion site of a pacemaker that began 3 months after subcutaneous implantation in the abdomen to stimulate the right obturator nerve. The pain was not accompanied by inflammatory signs or skin lesions, although it worsened after 9 months. A blood test revealed elevated C-reactive protein and neutrophilia. Cultures taken from the area were negative, and computed tomography and ultrasound scans of the soft tissue were normal. However, the pacemaker was removed owing to possible infection, and empiric antibiotic treatment was started. A new pacemaker was inserted, and 1 month later the patient presented severe pain, erythema, and surface heat. The pacemaker was removed definitively. Assessment at our allergy department 8 days later revealed that the local erythema and discomfort had resolved. The patient had no history of drug allergy or atopic or contact dermatitis and no other medical history of note.

A full work-up was performed with the T.R.U.E. test standard series (Smartpractice Denmark ApS), reagents of the pacemaker supplied by manufacturer (Medtronic), and other silicones and metals (Table).

Table. Results of Patch Testing^a

	48 h	96 h	Control Patient (48 and 96 h)
Medtronic pacemaker components			
A: Silicone medical adhesive A	(+++)	(++)	(-)
B: Silicone rubber (MDX-70 peroxide cured)	(++)	(+)	(-)
C: Silicone rubber (ETR-50 platinum-cured)	(++)	(+)	(-)
D: Silicone rubber (Med 4719 platinum-cured)	(++)	(+)	(-)
E: Polysulfone (amber)	(-)	(-)	(-)
F: Polyurethane (75D)	(-)	(-)	(-)
G: Polysulfone (beige)	(-)	(-)	(-)
H: Polyurethane (80A)	(-)	(-)	(-)
Metals, components of pacemaker and electrode			
1: Parylene-coated titanium	(-)	(-)	
2: Barium sulfate-filled silicone rubber	(+++)	(++)	
3: Titanium	(-)	(-)	
4: Platinum-iridium	(-)	(-)	
Silicones used in modeling and medical applications			
5: Glazier's silicone	(++)	(+)	
6: White silicone mold	(++)	(+)	
7: Transparent silicone mold	(++)	(+)	
8: Medical silicone (urinary catheter)	(-)	(-)	
T.R.U.E. test 3 panels			
Catalyzers			
Platinum (hexachloride) (in vaseline)	(-)	(-)	
Palladium (chloride) (in vaseline)	-	-	
Tin (metallic) (in vaseline)	-	-	
Rhodium (trichloride) (in vaseline)	++	++	

^aThe components were analyzed in physiological saline solution unless otherwise indicated.

We obtained positive results with rhodium and the 4 types of silicone used in the pacemaker, of which only 1 was rhodium-catalyzed (Silicone Medical Adhesive A).

Analysis of a biopsy specimen taken from the area affected by the application of a 2% rhodium patch confirmed contact dermatitis, which manifested as a minimal epidermal focus of spongiosis, lymphocytic exocytosis, and isolated necrotic keratinocytes. A minimal superficial perivascular lymphocytic infiltrate was also observed. Morphological changes were minimal and may be attributable to contact dermatitis.

Based on these data, we made a diagnosis of contact dermatitis induced by silicone and rhodium, although we cannot completely rule out the possibility of contamination of the platinum or silicones by rhodium.

Silicones are polymers of polysiloxane, which comprises chains of alternating silicon atoms and oxygen atoms, to which organic and inorganic groups can be added, thus providing them with various properties. Given their physicochemical characteristics, allergenicity is very low. Silicones are polymerized using catalysts such as transition metals (tin, platinum, palladium, or rhodium) and organic substances (eg, peroxide), which have known allergenicity.

Silicones are widely used in sectors such as health care, industry, transport, technology, and cosmetics, as well as in products used in the home. Cosmetics and home products are of great interest in allergy, because the increasingly frequent use of silicones means that they may be involved in hypersensitivity reactions.

Rhodium is a transition metal belonging to the platinum group that usually contaminates platinum. A scarce substance, it is used mainly as a catalyst in some platinum alloys; in its galvanized form, it is common in jewelry, electrical contacts, and mirrors. Hypersensitivity skin reactions due to rhodium are rare and occur mainly in the workplace [5,6].

The few reports of adverse reactions to silicones include foreign body reaction, skin necrosis, local pain, and, even more rarely, contact dermatitis [1-4,7-9]. The most common causes of adverse reactions to pacemakers are local infections, although in the absence of fever or other signs of infection (negative cultures, no leukocytosis), the possibility of contact hypersensitivity to its components should be considered.

Although rare, hypersensitivity to silicone should be taken into account in patients with pacemakers who experience contact dermatitis.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Previous Presentations

This study was presented as a poster at the XXIX National Congress of the Spanish Society of Allergology.

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Esther Lourdes Pérez González

Calle Manuel de Falla N° 1
28222 Majadahonda (Madrid)
Spain

E-mail: lourdesspg@gmail.com

Stevens-Johnson Syndrome After Influenza Vaccine Injection

Oda T, Sawada Y, Okada E, Yamaguchi T, Ohmori S, Haruyama S, Yoshioka M, Nakamura M

Department of Dermatology, University of Occupational and Environmental Health, Kitakyushu City, Fukuoka, Japan

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Key words: Stevens-Johnson syndrome. Influenza vaccine.

Palabras clave: Síndrome de Stevens-Johnson. Vacuna frente a la gripe.

Influenza vaccine is widely used for the prevention of seasonal influenza virus infection. Several cutaneous allergic reactions to influenza vaccine have been reported [1,2]. We report a case of Stevens-Johnson syndrome after administration of influenza vaccine.

A 75-year-old man had received influenza vaccine for prevention of seasonal influenza infection every year since 2014. He reported erythematous papules 2 days after receiving his injection in 2015. In 2016, he noticed erythema on his trunk and extremities 1 day after administration of the vaccine and developed lesions of the eye and oral mucosa 2 days later. He was referred to our department for evaluation of the skin eruption. He had taken no other medication and was not allergic to egg. Physical examination revealed annular erythema on the trunk (Figure, A) and a mucosal lesion in the oral cavity (Figure, B) and eyelids (Figure, C). The Nikolsky sign was positive. The patient had a high fever (37.7°C). A skin biopsy taken from the erythema on the trunk revealed dyskeratotic keratinocytes in the epidermis and lymphocytic infiltration in the epidermis and dermis (Figure, D). Furthermore, the dermis was infiltrated by eosinophils. The laboratory examination ruled out infection by herpes simplex virus, Epstein-Barr virus, and mycoplasma. Serum antidesmograin-1 or -3 antibody and bacterial swab culture from the skin erosion were negative. The

white blood cell count was slightly elevated. We diagnosed the skin eruption as Stevens-Johnson syndrome due to influenza vaccine. The patient received oral prednisolone (30 mg/d), and the eruption resolved completely 1 month after treatment. Because we could not obtain the patient's consent for patch testing using influenza vaccine, we performed patch testing using additives in the vaccine, which included formalin and thiomersal. However, the result of patch testing with these agents was negative. Therefore, we hypothesize that the patient developed Stevens-Johnson syndrome to the influenza vaccine itself.

Cutaneous adverse events reported after influenza vaccine injection include vasculitis [1] and lichenoid drug eruptions [2]. One case of Stevens-Johnson syndrome has been reported after combination treatment with influenza vaccine and flucloxacillin [3]. However, the case we report is the first case of Stevens-Johnson syndrome after influenza vaccine monotherapy. A vaccine consists of the vaccine itself and additives. In the present case, patch testing indicated that additives such as formalin and thiomersal were not responsible for the cutaneous adverse reaction. Therefore, this patient may have experienced a hypersensitivity reaction to the seasonal influenza vaccine itself, leading to Stevens-Johnson syndrome. Because the patient first developed the skin eruption after the 2015 seasonal influenza vaccine injection, a common antigen in the 2014 and 2015 seasonal influenza vaccine could have been the causative agent. We speculated that a causative agent might be A/California/7/2009(X-179A)(H1N1)pdm09, which was a common antigen in seasonal influenza vaccine from 2014 to 2016.

Additives in vaccine have been known to cause drug eruptions [4,5]. Thiomersal is a representative additive in vaccine and is used as a preservative in various presentations. It has been reported to be a possible causative agent for vaccine-induced drug eruptions [4]. The recently developed thiomersal-free influenza vaccine should prove beneficial in patients who experience hypersensitivity reaction to thiomersal.

Clinicians should bear in mind that not only the vaccine but also additives in the vaccine can cause vaccine-related drug eruption. Because influenza vaccine-induced cutaneous adverse events are relatively rare, further investigation is necessary to clarify their pathogenesis.



Figure. Clinical manifestations and histological examination. Annular erythema on the trunk (A), oral mucosa (B), and eyelid mucosa (C). D, Histology showing a dyskeratotic cell in the epidermis and lymphocytic infiltration in the epidermis and dermis.

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Yu Sawada

Department of Dermatology
University of Occupational and Environmental Health
1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan
E-mail: long-ago@med.uoeh-u.ac.jp