Strongyloidiasis: An Emerging Infectious Disease That Simulates Allergic Diseases

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Strongyloides stercoralis is an intestinal parasitic nematode that is present around the world and endemic in rural areas with tropical or subtropical climates [1]. In Spain, cases of infection have been described along the Mediterranean coast [2], in Zaragoza [3], and in southern Galicia [4]. A risk factor for infection in our country is agricultural work in wetlands or rice fields [2].

We present the case of a nonsmoking 27-year-old man of Bolivian origin who reported nonseasonal symptoms of 1-year duration consisting of expectoration that caused coughing every morning but was nonproductive throughout the rest of the day. He also experienced episodes of exacerbated symptoms with an intensely itchy throat and a feeling of mucus in the throat, although he reported that it primarily produced saliva. There was no fever. He was referred to our department to rule out an allergic cause.

Laboratory tests showed a total immunoglobulin (Ig) E level of 5000 kU/L and eosinophilia (700 cells/μL); serum determinations of specific IgE antibodies were positive for dust mites (*Dermatophagoides*) (1.42 kU/L), Poaceae (*Cynodon dactylon*, 7.82 kU/L; *Phleum pratense*, 4.46 kU/L), *Parietaria judaica* (3.12 kU/L), and *Olea europaea* (3.12 kU/L), but negative for dog and cat dander and *Alternaria alternata*. The patient reported that his symptoms had not improved despite daily treatment with oral antihistamines (ebastine 20 mg) for several months and inhaled budesonide at 400 μg every 6 hours, which had been prescribed just 24 hours before his first visit.

Due to the high levels of total IgE and the presence of eosinophilia, we ordered a stool test for parasites, and added omeprazole 40 mg per day to budesonide every 8 hours to evaluate its therapeutic effect. On receiving the request for a stool test in a Bolivian patient with such high IgE levels, the microbiology laboratory asked for new stool samples without preservatives to test for *S stercoralis* larvae. Several rhabditoid larvae were observed in the stools by optical microscopy and abundant filariform larvae were found in the 24-hour agar-plate culture, which is a more sensitive method in which larger samples are analyzed. When informed of the positive results for strongyloidiasis, we immediately withdrew the inhaled budesonide and referred the patient for urgent evaluation at the International Medicine Unit of the Infectious Diseases Department at Hospital General Universitario de Valencia to initiate treatment (ivermectin at 200 μg/kg/d for 2 days).

On questioning the patient to find a possible source of exposure to the parasite, he reported having walked barefoot as a child through agricultural areas and standing water.

Autoinfection with *S stercoralis* is a chronic disease that can last for decades if the infected person's immune system is intact [1,5]. It can be clinically invisible (in up to half of cases the only sign is fluctuating eosinophilia) or it may manifest itself through symptoms that appear only when the larvae migrate. These include cutaneous symptoms (burning, urticaria, and pruritus in the area penetrated by the larvae); gastrointestinal symptoms (epigastralgia, indigestion, intermittent mucous diarrhea, chronic malabsorption); and respiratory symptoms (cough, fever, hoarseness, and bronchospasm) [1,4,5].

Humans become infected when the larva penetrates the skin of the hands, feet, or back in its filariform state (L3). It then enters the circulatory system and reaches the lungs, from where it is coughed up and swallowed. Once in the intestines it matures into a parthenogenetic female that lays eggs that hatch into rhabditiform larvae (L1) that are excreted in feces to complete their cycle in the soil, where they await another host [4]. Some L1 larvae can mature into L3 larvae and, through the colonic mucosa or the perianal skin, cause endogenous autoinfection that produces few symptoms [1].

Diagnosis is established by detection of rhabditoid larvae in stools, and filariform larvae can be seen in a 24-hour agar culture. The parasite load is low, and larval expulsion to the outside environment is very irregular. A simple stool test can fail in up to 70% of cases [5,6].

Depending on the state of infection, the parasite can be found, in all stages of its lifecycle, in bronchoalveolar lavage fluid [3] cerebrospinal fluid, urine, blood, gastric fluid, and peritoneal lavage fluid [6].

A number of enzyme-linked immunosorbent assays have recently proven to be useful for diagnosis and posttreatment evaluation, although they are less effective in patients with hematological malignancies or HTLV-1 infection [7]; they also show cross-reactivity with hookworms, filariae, and schistosomes [6].

Most cases of severe *S stercoralis* infection occur in patients who have been taking corticosteroids for over a year [5,7,8]. It has been reported that corticosteroid doses of over 0.3 mg/kg/d can facilitate the loss of T lymphocytes from the circulation, thereby disrupting their ability to reach the site of infection [6]. It is also known that corticosteroids accelerate the maturation of *S Stercoralis* larvae in the intestine, suppress eosinophilia [7], and reduce local inflammation, thus eliminating another barrier to the migration of this parasite [6].

A considerable number of cases of disseminated strongyloidiasis reported in the literature have been in patients with chronic obstructive pulmonary disease. In such cases, the respiratory symptoms of strongyloidiasis can be masked by the pre-existing bronchial disease, leading to delays in diagnosis and in initiation of appropriate treatment [9].

Because of current immigration trends and the persistence of this parasitic infestation, which can last for years or even decades, we believe that it is important to alert allergy specialists in settings such as ours to the possibility of an increase in cases. The frequent administration of corticosteroids in patients treated in our offices increases the risk of hyperinfection, a severe complication associated with a mortality of around 80% [7,10].

We stress the importance of testing for strongyloidiasis when eosinophilia is observed in a patient who is from a disease-endemic area or who has a history of working in wetland areas [2,3,8]. Particular attention is required in patients who require immunosuppressive or corticosteroid therapy [10].

As a microbiological diagnosis cannot be made with standard stool examination, the laboratory should be advised of the possibility of strongyloidiasis and sent stool samples without preservatives or refrigeration [4,5]; strongyloides serology should also be ordered. If the stool studies are negative and clinical suspicion persists, the patient should be followed up with repeated stool studies, especially when serology is positive [5,7].

References

1. Genta RM. Global prevalence of strongyloidiasis: clinical review with epidemiologic insights into the prevention of disseminated disease. Rev Infect Dis. 1989; 11:755-67.
2. Roman-Sánchez P, Pastor Guzman A, Moreno Guillem S, Igual Adell R, Suner Generoso S, Tornero Estébanez C. High prevalence of Strongyloides stercoralis among farm workers on the Mediterranean coast of Spain: analysis of the predictive factors of infection in developed countries. Am J Trop Med Hyg. 2003; 69: 336-40.
3. Mayayo E, Gómez-Araal V, Azua-Romero J, Capilla J, Mayayo R. Strongyloides stercoralis infection mimicking a malignant tumor in a non-immunocompromised patient. Diagnosis by broncoalveolar cytology. J Clin Pathol. 2005; 58(4): 420-2.
4. Martínez-Vázquez C, González-Medinero G, Núñez M, Pérez S, García-Fernández JM, Gimena B. Strongyloides stercoralis en el sur de Galicia. An Med Interna (Mad), 2003; 20(9): 477-9.
5. Segarra-Newnham M. Manifestations, diagnosis and treatment of Strongyloides stercoralis infection. Ann Pharmacother. 2007; 41(12): 1992-2001.
6. Siddiqui AA, Berk SL. Diagnosis of Strongyloides infection. Clin Infection. 2001; 33: 1040-7.
7. Marcos LA, Terashima A, Dupont HL, Gotuzzo E. Strongyloides hyperinfection syndrome: an emerging global infectious disease. Trans R Soc Med Hyg. 2008; 102 (4): 314-8.
8. Pardo Moreno G, Rodríguez Rodríguez R, Campillos Paez MT. Strongyloides Stercoralis: factores de riesgo para estrongiloidiasis. Med clin (Barc). 2003; 121 (7): 662-4.
9. Pretel Serrano L, Page del Pozo MA, Ramos Guevara MR, Ramos Rincón JM, Martínez Toldos MC, Herrero Huerta F. Infestación por Strongyloides stercoralis en pacientes con enfermedad pulmonar obstructiva crónica en la Vega del Segura (Murcia). Presentación de tres casos. Rev Clin Esp. 2001; 201:109-10.
10. Llagunes, J. Mateo, E. Peña, J.J. Carmona, P. de Andrés, J. Hiperinfección por Strongyloides stercoralis. Med Intensiva. 2010; 34 (5):353-6.

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Urticaria-Angioedema Due to Carboxymethylcellulose Eye Drops

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Carboxymethylcellulose (CMC) is a synthetic polymer, soluble in water, in which CH_2COOH groups replace glucose units on the cellulose chain through an ether bond. Its molecular weight ranges from 21 kDa (low-density CMC) to 500 kDa (high-density CMC). The product is the sodium salt of carboxylic acid. CMC is formed from cellulose (the main polysaccharide constituent of wood and all plant structures) through a reaction between alkaline cellulose and chlorine sodium acetate. It can be produced commercially by the chemical modification of wood. The commercial product is soluble in water and comes as colorless, odorless powder or granules.

CMC has very diverse uses. It is used mainly as a thickening agent, but also as a filler, dietary fiber, an anti-clumping agent, and an emulsifier. It is found in detergents, soaps, cosmetics and pharmaceutical products, and foodstuffs (in which it is used as a thickener, a binder, a suspension agent, and a stabilizer); it is also used in paper, cardboard, and textile manufacturing.

There have been reports of anaphylactic shock following the administration of CMC in corticosteroid injections [1-4] and barium sulfate suspensions [5,6]. CMC is also used as a tear-replacement product in the treatment of dry eye syndrome [7] and has been known to cause contact allergic dermatitis in this setting [8]. To the best of our knowledge, however, there have been no reports of a type 1 hypersensitivity reaction to CMC in an ophthalmic solution.

We present the case of a 44-year-old man, a sweeper by profession, with a history of high blood pressure under treatment with ramipril. Several weeks earlier, he had been prescribed an eye drop called Viscofresh (CMC) to treat a sore eye. Immediately after applying the drops, he developed intense conjunctival erythema and bilateral periocular edema with urticarial lesions on the face and trunk. He went to the emergency room, where he was administered methylprednisolone and dexchlorpheniramine intramuscularly and instructed to continue oral treatment for 5 days, until complete remission of the clinical manifestations.

The patient commented that 6 months before the reaction he had developed generalized urticaria, without angioedema or respiratory distress, immediately after a lumbar epidural infiltration with Trigon Depot (triamcinolone + Tween 80 + CMC + benzyl alcohol) and bupivacaine to treat left lumbosciatic pain (discopathy L5-S1). He had been treated with intramuscular methylprednisolone and dexchlorpheniramine, and responded well, although some of the lesions lasted for 2 days.

In our evaluation of the patient, we performed skin prick tests (SPTs) with Viscofresh drops (CMC) at a concentration of 5 mg/mL and Trigon Depot at a concentration of 40 mg/mL. The results were positive in both cases (and negative in 5 controls). SPTs with Tween 80 and benzyl alcohol were negative, as were an oral challenge with triamcinolone and a subcutaneous challenge with bupivacaine

We used the dot blot technique described by Towbin et al [9] to test for the presence of specific immunoglobulin (Ig) E to CMC. This technique, which is used to detect, analyze, and identify proteins, differs from the Western blot technique in that protein samples are not separated electrophoretically but are spotted directly onto a membrane or paper substrate through circular templates. Once the samples had been spotted onto the polyvinyl difluoride membrane at the center of the grid, the membrane was left to dry and nonspecific sites were blocked by soaking in 1% bovine serum albumin in phosphate-buffered serum (PBS)-Tween 0.5%. The primary antibody (patient serum) and then the secondary conjugated antibody (anti-IgE) were incubated with an enzymatic marker that emits a signal, collected on photographic film, when it comes in contact with another reagent (in our case, a luminescent agent).

The dot blot showed that the samples of Viscofresh (CMC) undiluted and diluted at concentrations of 1:2, 1:4, and 1:10 in 1% BSA in PBS-Tween 0.5% reacted with the patient's serum, whereas there was no reaction with the atopic control serum (Figure). There was no recognition of serum when only blocking buffer was added to the dot (negative control for technique). We can therefore conclude that CMC was specifically recognized by the patient's serum.

We have presented a case of a patient who developed urticaria and angioedema following the administration of CMC as eye drops, and have proven, using SPTs and laboratory tests, that the reaction was IgE-mediated. It is worth noting that a

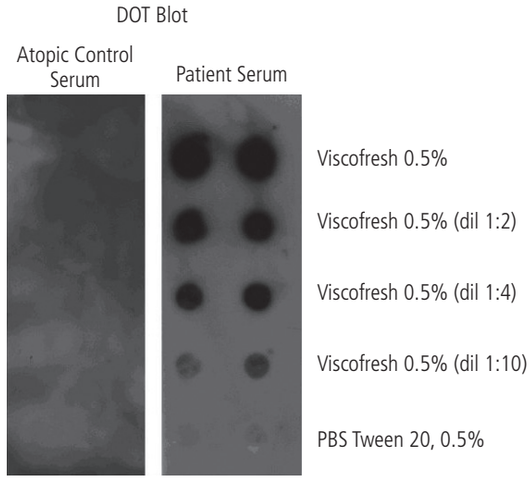

Figure. Dot Blot results. Patient and atopic control serum incubated with different concentrations of carboxymethylcellulose. Dil indicates dilution.

few months earlier, the patient had also developed a urticarial reaction to a corticosteroid injection containing sodium CMC.

To our knowledge, this is the first report of an IgE-mediated urticarial reaction after the administration of CMC as eye drops.

References

1. Patterson DL, Yunginger JW, Dunn WF, Jones RT, Hunt LW. Anaphylaxis induced by the carboxymethylcellulose component of injectable triamcinolone acetonide suspension (Kenalog). Ann Allergy Asthma Immunol. 1995;74(2):163-6.
2. Montoro J, Valero A, Elices A, Rubira N, Serra-Baldrich E, Amat P, Malet A. Anaphylactic shock after intra-articular injection of carboxymethylcellulose. Allergol Immunopathol (Madr). 2000;28(6):332-3.
3. Venturini M, Lobera T, del Pozo MD, González I, Blasco A. Immediate hypersensitivity to corticosteroids. J Investig Allergol Clin Immunol. 2006; 16(1):51-6.
4. Murrieta-Aguttes M, Michelen V, Leynadier F, Duarte-Risselin C, Halpern GM, Dry J. Systemic allergic reactions to corticosteroids. J Asthma.1991;28(5):329-39.
5. Muroi N, Nishibori M, Fujii T, Yamagata M, Hosoi S, Nakaya N, Saeki K, Henmi K. Anaphylaxis from the carboxymethylcellulose component of barium sulfate suspension. N Engl J Med. 1997 Oct 30;337(18):1275-7.
6. Dumond P, Franck P, Morisset M, Sainte Laudy J, Kanny G, Moneret-Vautrin DA. Pre-lethal anaphylaxis to carboxymethylcellulose confirmed by identification of specific IgE--review of the literature. Eur Ann Allergy Clin Immunol. 2009;41(6):171-6.
7. Bruix A, Adán A, Casaroli-Marano RP. Eficacia de la carboximetilcelulosa sódica para el tratamiento del síndrome del ojo seco. Arch Soc Esp Oftalmol. 2006; 81: 85-92.
8. Hernández Santana G, Heras Mendoza F, Martínez Tadeo JA, Matheu V-Conde-Salazar. Dermatitis alérgica de contacto por carboximetilcelulosa. In: Tópicos y Reacciones Tardías I. Sociedad Española de Alergología e Inmunología Clínica (SEAIC). Available from: http://www.postersessiononline.com/312191188_es/congresos/seaic2009/aula/-P_164_seaic2009.pdf. Symposium Internacional de alergia a medicamentos, 22-24 octubre de 2009, Logroño, Spain.
9. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A. 1979; 76(9): 4350-4.

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Salmonella Vertebral Osteitis and Sepsis in a Girl With Interferon Gamma Pathway Deficiency

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Palabras clave: Osteitis por salmonella, micobacteria, inmunodeficiencia primaria, interferón gamma.

Primary immunodeficiency diseases are caused by congenital defects in cellular, humoral, or molecular functions of the intricate immune system. More than 160 of these defects have been described, and at least 100 genes have been incriminated [1]. Mendelian susceptibility to mycobacterial disease (MSMD) is a clinically defined, genetically heterogeneous group of primary immunodeficiencies caused by mutations in genes that code for both components of the interferon (IFN) γ-interleukin 12/23 circuit between lymphocytes and macrophages [2,3] and the respiratory burst pathway [4], thereby disrupting the immune response to intracellular pathogens. Patients are otherwise quite healthy and only rarely develop other unusually severe bacterial, viral, fungal, or parasitic diseases [5]. In practically all cases, patients are vulnerable to mycobacteria; they can develop adverse reactions to the bacille Calmette-Guérin (BCG) vaccine and infections caused by environmental nontuberculous mycobacteria. One notable exception is non-typhi Salmonella, which causes disease in approximately 50% of these patients [2,5]. To date, 8 genes (*IFNGR1, IFNGR2, IL12B, IL12RB1, STAT1, IRF8, IKBKG*, and *CYBB*) and 16 genotypes have been identified as responsible for MSMD [2,3,4]. Nonetheless, the genetic etiology is unknown in approximately 50% of patients.

Here, we describe the case of a female preschool-age patient who did not react to the BCG vaccine but did develop severe disseminated *Salmonella* infection in association with vertebral osteitis treated with broad-spectrum antibiotics and surgical resection. Both *Mycobacterium tuberculosis* and Group D *Salmonella* were identified.

The patient was a 4-year-old Mexican girl born at term

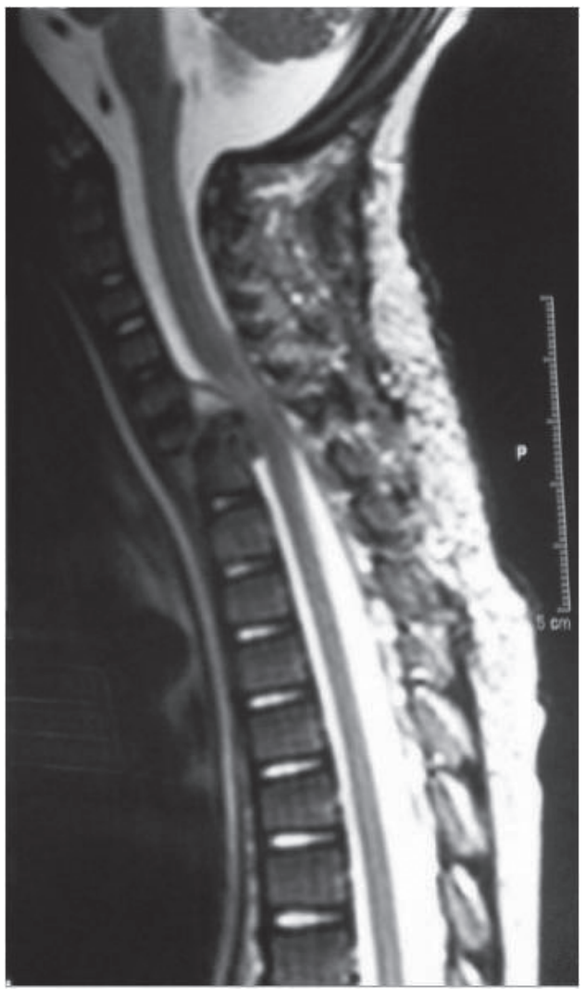

Figure. Computed tomography scan of the cervical vertebral column; sagittal view showing osteolytic lesions of vertebral bodies C7 and T1.

to nonconsanguineous parents in 2006. She had received the BCG vaccine (Pasteur substrain) as a newborn. At the age of 22 months, she presented with a 5-month history of neck spasticity and progressive descending weakness in her arms and legs. On physical examination, she was found to have left-sided torticollis and a small, firm, nontender posterior cervical mass. Neurological examination revealed intact cranial nerves and superficial sensitivity, but brisk tendon reflexes, as well as reduced muscular strength and a bilateral extensor plantar reflex. A pyramidal syndrome was noted, and the patient was admitted for further investigation.

Computed tomography of the neck revealed osteolytic lesions of vertebral bodies at C7 and T1 (Figure), suggestive of tuberculous osteitis of the spine (Pott's disease). The patient underwent an excisional biopsy of the cervical mass and a posterior corpectomy. Surgical specimens had nonspecific acute inflammation, with no evidence of granuloma formation. A polymerase chain reaction (PCR) was positive for Mycobacterium tuberculosis. Furthermore, Group D *Salmonella* was grown from surgical specimens and serial cultures from blood. A 4-drug antituberculosis regimen was started and endovenous ceftriaxone was administered for 6 weeks.

The laboratory workup revealed leukocytosis with neutrophilia. Serum immunoglobulins, complement levels, lymphocyte subpopulations, and the nitroblue tetrazolium (NBT) reduction assay were all normal. Superoxide production by lymphoblastoid (Epstein-Barr virus–transformed) cells obtained from the patient was also normal as measured by the cytochrome-c reduction assay. Baseline plasma IFN-γ was undetectable. Whole-blood activation assay with BCG, IL-12, and IFN-γ revealed normal interferon production in response to stimulation with BCG+IL-12; low but present IL-12p40 as measured by enzyme-linked immunosorbent assay; and a lack of IL-12p70 production in response to BCG+IFN-γ [6]. Partial *IFNGR* deficiency was suspected on the basis of the presence of IL-12 but a lack of response to appropriate stimuli.

The patient completed 9 months of antimycobacterial treatment and is currently alive and well. No other mycobacterial or opportunistic infections have been observed. She is not receiving oral prophylaxis with antibiotics or subcutaneous recombinant interferon gamma. Genetic analysis to further characterize the defect is currently being pursued.

We have described the case of a preschool-age girl with MSMD who developed vertebral osteitis and sepsis. Both *Salmonella* species and *Mycobacterium tuberculosis* were identified from surgical specimens, and good recovery was observed after the patient received a multidrug antibiotic regimen.

MSMD was probably first described clinically in 1951, and its first genetic etiology was identified in 1996 [7]. This group of immune defects has a high degree of allelic heterogeneity and exists as recessive, dominant, or X-linked inheritance patterns and in both partial and complete forms [2]. Contrary to previous reports of MSMD [2,5], our patient's history is remarkable in that she did not develop *Mycobacterium bovis* infection after administration of the BCG vaccine, and she first presented as a toddler with a severe, invasive, acute *Salmonella* infection, which apparently coexisted with a chronic insidious mycobacterial infection of the vertebrae caused by *Mycobacterium tuberculosis*.

Salmonellae are gram-negative, facultative anaerobic intracellular bacteria that usually cause foodborne gastrointestinal infections. Bacteremia develops in less than 5% of all patients with *Salmonella* gastroenteritis [8], and dissseminated infections are only rarely seen, except in patients with acquired immunosuppression (eg, human immunodeficiency virus, rheumatoid arthritis, diabetes mellitus, patients receiving tumor necrosis factor blockers), or congenital defects (eg, Sickle-cell disease, chronic granulomatous disease, hyper-IgM syndrome, MyD88, and NEMO deficiencies) [9]. Recurrent, persistent and severe invasive *Salmonella* infections are seen in about 50% of patients with MSMD, especially in defects affecting the IL-12/23 loop of the IL12/23-IFN-γ circuit [8], but also in *STAT1* deficiency [5]. In our patient, age at onset (2 years),

clinical presentation (multifocal vertebral osteitis), and clinical outcome (good response to antibiotics and full recovery) all suggest a partial defect in the *IFNGR1*, *IFNGR2*, or *STAT1* genes. However, MSMD is defined clinically, and novel genetic etiologies are being discovered at a fast rate.

Salmonella vertebral osteomyelitis presents with several features similar to those of pyogenic vertebral osteitis due to other agents, including mycobacteria. Depending on the context, for example, in regions of the world where tuberculosis is endemic, *Salmonella* osteitis of the vertebrae can be mistaken for tuberculosis [10] and treated inadequately. When faced with vertebral osteitic lesions, thus, especially in a patient suspected to have MSMD, histopathology images and cultures are indispensable for the clinician to distinguish between salmonellal and mycobacterial etiologies.

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References

1. Alcais A, Abel L, Casanova JL. Human genetics of infectious diseases: between proof of principle and paradigm. J Clin Invest. 2009; 119 (9):2506-14.
2. Al-Muhsen S, Casanova JL. The genetic heterogeneity of mendelian susceptibility to mycobacterial diseases. J Allergy Clin Immunol. 2008; 122 (6):1043-51; quiz 1052-3.
3. Hambleton S, Salem S, Bustamante J, Bigley V, Boisson-Dupuis S, Azevedo J, Fortin A, Haniffa M, Ceron-Gutierrez L, Bacon CM, Menon G, Trouillet C, McDonald D, Carey P, Ginhoux F, Alsina L, Zumwalt TJ, Kong XF, Kumararatne D, Butler K, Hubeau M, Feinberg J, Al-Muhsen S, Cant A, Abel L, Chaussabel D, Doffinger R, Talesnik E, Grumach A, Duarte A, Abarca K, Moraes-Vasconcelos D, Burk D, Berghuis A, Geissmann F, Collin M, Casanova JL, Gros P. IRF8 mutations and human dendritic-cell immunodeficiency. N Engl J Med. 2011; 365 (2):127-38.
4. Bustamante J, Arias AA, Vogt G, Picard C, Galicia LB, Prando C, Grant AV, Marchal CC, Hubeau M, Chapgier A, de Beaucoudrey L, Puel A, Feinberg J, Valinetz E, Janniere L, Besse C, Boland A, Brisseau JM, Blanche S, Lortholary O, Fieschi C, Emile JF, Boisson-Dupuis S, Al-Muhsen S, Woda B, Newburger PE, Condino-Neto A, Dinauer MC, Abel L, Casanova JL. Germline CYBB mutations that selectively affect macrophages in kindreds with X-linked predisposition to tuberculous mycobacterial disease. Nat Immunol. 2011; 12 (3):213-21.
5. Filipe-Santos O, Bustamante J, Chapgier A, Vogt G, de Beaucoudrey L, Feinberg J, Jouanguy E, Boisson-Dupuis S, Fieschi C, Picard C, Casanova JL. Inborn errors of IL-12/23- and IFN-gamma-mediated immunity: molecular, cellular, and clinical features. Semin Immunol. 2006; 18 (6):347-61.
6. Feinberg J, Fieschi C, Doffinger R, Feinberg M, Leclerc T, Boisson-Dupuis S, Picard C, Bustamante J, Chapgier A, Filipe-Santos O, Ku CL, de Beaucoudrey L, Reichenbach J, Antoni G, Balde R, Alcais A, Casanova JL. Bacillus Calmette Guerin triggers the IL-12/IFN-gamma axis by an IRAK-4- and NEMO-dependent, non-cognate interaction between monocytes, NK, and T lymphocytes. Eur J Immunol. 2004; 34 (11):3276-84.
7. Jouanguy E, Altare F, Lamhamedi S, Revy P, Emile JF, Newport M, Levin M, Blanche S, Seboun E, Fischer A, Casanova JL. Interferon-gamma-receptor deficiency in an infant with fatal bacille Calmette-Guerin infection. N Engl J Med. 1996; 335 (26):1956-61.
8. Fieschi C, Casanova JL. The role of interleukin-12 in human infectious diseases: only a faint signature. Eur J Immunol. 2003; 33 (6):1461-4.
9. Freeman AF, Holland SM. Persistent bacterial infections and primary immune disorders. Curr Opin Microbiol. 2007; 10 (1):70-5.
10. Laloum E, Zeller V, Graff W, Aerts J, Chazerain P, Mamoudy P, Ziza JM, Desplaces N. Salmonella typhi osteitis can mimic tuberculosis. A report of three cases. Joint Bone Spine. 2005; 72 (2):171-4.

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Skin Reactions to Gadolinium-Based Contrast Media

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Palabras clave: Contrastes de gadolinio. Alergia a medicamentos. Tests inmunológicos. Imagen de Resonancia Magnética. Reacciones de Hipersensibilidad.

Gadolinium-based contrast media are used for magnetic resonance imaging (MRI). The most common agents are gadopentetate dimeglumine (Magnevist), gadobutrol (Gadovist), gadobenate dimeglumine (MultiHance), gadoxetate disodium (Primovist), and gadoteridol (Prohance).

Adverse reactions are uncommon, with prevalence ranging between 0.066% and 1.47%. The most common types of adverse reactions are hypersensitivity reactions, such as erythema and urticaria; anaphylaxis occurs in just 0.01% of cases.

We report 2 cases of hypersensitivity to gadolinium-based contrast agents, including that of a patient in whom positive skin test results suggest a possible immunoglobulin (Ig) E-mediated mechanism.

The first case involved a 17-year-old black girl with a history of albinism, hypothyroidism, and cervical squamous cell carcinoma treated with surgery. She required 6-monthly MRI for monitoring of her tumor. In July 2009, she developed generalized urticaria and pruritus immediately after MRI with gadopentetate dimeglumine. She was treated with antihistamines and corticosteroids, and her condition improved in 2 to 3 hours. She had tolerated the same procedure with the same contrast agent several times between January 2008 and July 2009.

The second case involved a 4-year-old North African girl with a history of low-grade chiasmatic-hypothalamic glioma that had been treated with chemotherapy. She also required 6-monthly MRI to monitor her glioma. In July 2010 she developed a generalized rash with no other symptoms immediately after MRI with gadoteridol. The rash resolved in minutes with antihistamines. She had previously tolerated MRI with gadopentetate dimeglumine.

Skin prick tests (SPT, 1:1) and intradermal tests (IDT, 1:10) were performed with the agents implicated in the reactions and several alternatives: gadopentetate dimeglumine, gadobutrol, gadobenate dimeglumine, gadoteridol, and gadoxetate disodium.

SPT was performed first. When the results were negative, IDT was performed with contrast agents from the same series. Histamine and normal saline were used as positive and negative controls, respectively, and a wheal of greater than 3 mm was considered positive. The tests were also performed in a group of 10 nonatopic individuals and an atopic control group, with negative results in all cases.

Challenge testing was performed, following signed informed consent from the parents, with the injection of an alternative gadolinium contrast (single dose according to the patient's weight) diluted in 250 cc of saline.

In case 1, the SPT and IDT results were negative for all the gadolinium-based contrast media tested. As the patient needed to continue monitoring with MRI, we performed a controlled exposure test with gadoteridol, with negative results. We did not test gadopentetate dimeglumine, the agent that had been implicated in the reaction, because it had been withdrawn from the hospital.

In case 2, the SPT results were negative for all the agents tested. The IDT results (with immediate reading) were negative for gadopentetate dimeglumine and gadobenate dimeglumine and positive for gadoteridol, gadobutrol, and gadoxetate disodium (Figure). For the challenge test, we used gadobenate dimeglumine, which had yielded negative SPT and IDT results. The girl tolerated the contrast without problems.

Hypersensitivity reactions with gadolinium-based contrast agents are rare, with very few cases described in the literature. The first case dates from 1995, when Jordan et al [1] reported anaphylactic shock to gadopentetate dimeglumine. The next case was reported in 2005 by Rahman et al [2], who described a case of anaphylaxis following initial exposure to a gadolinium-based contrast. Kalogeromitros et al [3] described a similar case after MRI with gadobenate dimeglumine, which produced a positive IDT response. Hasdenteufel et al [4] reported 2 cases of anaphylactic shock after initial exposure to gadopentetate dimeglumine in MRI. In both cases, the SPT and IDT results were positive for the agent involved. In 2009, Watson et al [5] described the case of a 58-year-old woman who developed anaphylactic shock after exposure to a gadolinium-based contrast agent, but an allergy study was not performed because of the poor baseline condition of the patient. Finally, Galera et al [6] described 2 cases of anaphylaxis after the administration of gadoteridol and gadobenate dimeglumine. In both cases, the skin tests were performed with the contrast involved in the reaction and a series of alternatives. The patient exposed to gadobenate dimeglumine had a positive IDT to this agent, while the other patient had a positive SPT to gadoteridol (the agent implicated in the reaction) and a positive IDT to the other agents tested. The positive skin test results suggest that dilutions do not cause a reaction and indicate the possibility

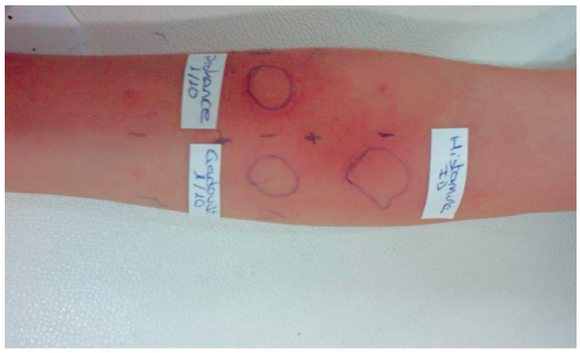

Figure. Figure Positive gadolinium prick test.

of multiple sensitization, highlighting the need for assessing cross-reactivity between contrast agents.

None of the cases published to date have suggested an alternative for patients who need to undergo gadolinium MRI in the future. In our first case, the skin tests were negative but the suggestive clinical reaction and the need for the patient to continue MRI monitoring led us to perform a challenge test with an alternative agent. In the second case, the positive IDT results may indicate an IgE-mediated mechanism. As the skin test results were positive for several gadolinium-based contrasts, we considered the possibility of an alternative radiological method, but this was not possible because MRI was necessary to monitor the patient's disease. We therefore performed a challenge with gadobenic acid as this had produced a negative IDT response. This contrast agent is used to display liver structures, although it can be used for cranial structures in the first phase of intravenous infusion. The patient tolerated the agent well.

Our findings for this second case are consistent with those described by Galera et al [6] and we consider that a positive skin test result for several gadolinium-based contrast agents might suggest cross-reactivity. We emphasize the importance of challenge testing with alternative contrasts, where possible, as this may be of vital importance for patients who require radiological monitoring. Further studies are necessary to elucidate the mechanisms potentially involved in these reactions.

References

1. Jordan RM, Mintz D. Fatal Reaction to gadopentetate dimeglumine. AJR 1995;164:743-44.
2. Rahman SL, Harbinson MT, Mohiaddin R, Pennell DJ. Acute allergic reaction upon first exposure to gadolinium-DTPA: a case report. J Cardiovasc Magn Reson. 2005;5:849-51.
3. Watson C, Benouni S, Gibbon G, Klaustermeyer W. Severe anaphylactoid shock secondary to gadolinium contrast Media. Ann Allergy Asthma Immunol. 2009;103:359-60.
4. Kalogeromitros DC, Makris MP, Aggelides XS, Spanoudaki N, Gregoriu SG, Augerinou G, Rigopoulos DG. Anaphylaxis to gadobenate dimeglumine: a case report. Int Arch Allergy Immunol. 2007;144:150-4.
5. Hasdenteufel F, Luyasu S, Renaudin JM, Paquay JL, Carbutti G, Beaudouin E, Moneret-Vautrin DA, Kanny G. Anaphylactic shock after first exposure to gadoterate meglumine: two case reports documented by positive allergy assessment. J Allergy Clin Immunol. 2008;121:527-8.
6. Galera C, Pur L, Cavigioli S, Bousquet PJ, Demoly P. Gadoteridol-induced anaphylaxis - not a class allergy. Allergy. 2010;65:132-4.

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Occupational Asthma Due to Western Red Cedar in a Guitar Maker

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Key words: Guitar maker. Occupational asthma. Western red cedar.

Palabras clave: Guitarrero. Asma ocupacional. Madera de cedro rojo del Canadá

Occupational asthma caused by exposure to western red cedar (WRC) (*Thuja plicata*) has been described in the sawmill industries of the Pacific Northwest [1] but no cases have been reported to date in Europe. One of the nonvolatile compounds isolated from WRC, plicatic acid, has been identified as the agent responsible for asthma due to WRC [2].

WRC is used in the manufacture of guitars due to its elasticity and tone. Although this wood was introduced to the Spanish guitar industry in the 1970s, no cases of occupational asthma among guitar makers have been reported to date.

We report the case of a 22-year-old male smoker who had worked making hand-crafted guitars since 2006; his job involved sanding and shaping wood, especially WRC. In September 2009 he developed a respiratory infection followed by dry cough, dyspnea, and wheezing. Despite treatment with salmeterol/fluticasone (50/500 μg twice daily) the dyspnea persisted, predominantly at night. He experienced a marked improvement at weekends but his symptoms worsened on Monday nights. His condition worsened progressively for 3 months and he required treatment for severe dyspnea and cough in the emergency department on 3 occasions.

A chest radiograph and blood tests were normal except for eosinophilia (850 eosinophils/mm^3). Spirometric parameters were within normal limits, with a forced vital capacity (FVC) of 5.19 L (103% of predicted), a forced expiratory volume in the first second of FEV_1 of 4.10L (96%), and an FEV_1/FVC ratio of 75.3%. The bronchodilator test was negative. The methacholine inhalation test was positive (20% fall in FEV_1 from baseline [PC20] at 1 mg/mL) and the fraction of exhaled nitric oxide (Fe_{NO}) was 42 ppb. On evaluating the patient after he had been absent from work for 15 days, the methacholine test was negative (PC_{20} >16 mg/mL) and Fe_{NO} was 30 ppb. Total serum immunoglobulin (Ig) E was 146 kU/L.

Skin prick tests with common aeroallergens and commercial wood extracts (Bial-Aristegui,) were negative, as was a prick test with a sawdust extract of WRC (10% wt/vol). An extract of WRC free of volatile components was prepared [3] at 2.5 mg/mL.

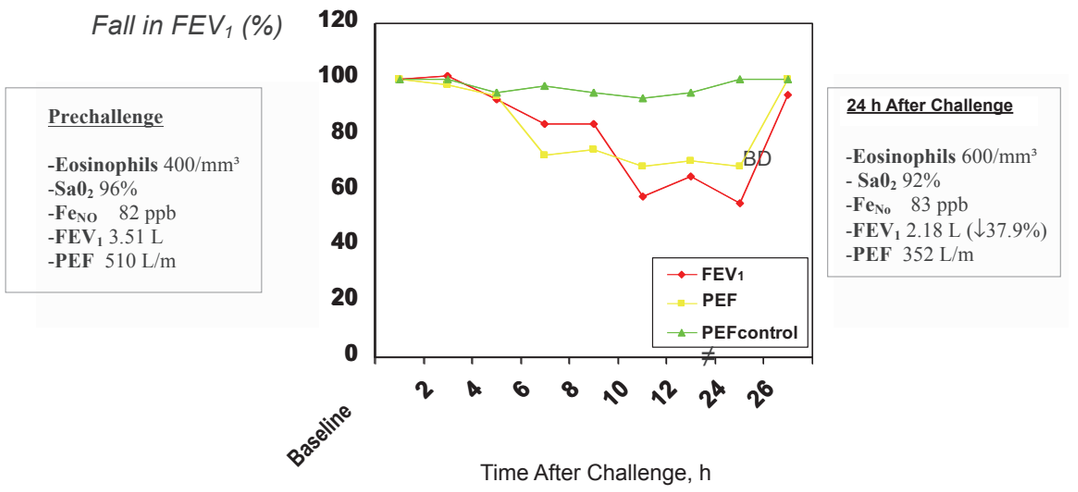

Figure. Specific inhalation challenge with western red cedar at 2.5 mg/mL in the patient and in a control. SaO2 indicates oxygen saturation by pulse oximetry; Fe_{NO}, exhaled nitric oxide; FEV_1, forced expiratory volume in the first second; PEF, peak expiratory flow.

A prick test with this extract was negative in the patient and in 5 nonexposed controls.

After the patient had stopped working for 4 weeks and was free of symptoms, we performed a specific inhalation challenge (SIC) [3] with WRC at 2.5 mg/mL and with a control solution using a DeVilbiss 646 nebulizer (DeVilbiss) at tidal breathing for 1 to 8 minutes. Spirometry was performed at 5, 10, 20, 30, 40, 60 and 120 minutes after the challenge. From that moment, peak expiratory flow (PEF) and FEV_1 measurements were performed hourly with an electronic PEF/FEV_1 meter (Vitalograph) for 24 hours, except during sleep. The SIC elicited an isolated late asthmatic response, with a maximum fall in FEV_1 of 40% 10 hours after the challenge. An increase in peripheral blood eosinophils and a decrease in oxygen saturation were observed 24 hours after the SIC, but no significant changes were observed in Fe_{NO} (Figure). The methacholine inhalation test could not be repeated 24 hours after the SIC because the patient still had airflow obstruction and required treatment with inhaled bronchodilators. No reaction was observed in a healthy nonexposed control.

A basophil activation test (Basotest) was performed with WRC extract at different concentrations (0.625, 1.25, 2.5 and 5 mg/mL) using whole blood obtained from the patient and a control [4]. A 2-fold increase in WRC-induced degranulation was observed in the patient compared to the control with WRC 5 mg/mL.

The patient was diagnosed with occupational asthma due to WRC. He quit his job and had no further exposure to WRC. Six months later he was asymptomatic.

The pathogenesis of occupational asthma due to WRC remains to be elucidated. Histamine and leukotrienes can be detected in bronchoalveolar lavage fluid after inhalation of plicatic acid. Peripheral blood basophils from patients with WRC-induced asthma release histamine with plicatic acid, but specific immunoglobulin (Ig) E to this compound is detected only in a small proportion of patients [5]. Because our patient had a late asthmatic response with peripheral blood eosinophilia (probably due to eosinophilic airway inflammation) and a positive basophil activation test after a challenge with WRC, we can assume the involvement of an immunologic mechanism.

References

1. Chan-Yeung M, Lam S, Koerner S. Clinical features and natural history of occupational asthma due to western red cedar (Thuja plicata). Am J Med. 1982;72:411-5.
2. Chan-Yeung M, Barton GM, MacLean L, Grzybowski S. Occupational asthma and rhinitis due to western red cedar. Am Rev Respir Dis. 1973;108:1094-102.
3. Chan-Yeung M, Barton GM, Mac Lean L, Grzybowski S. Bronchial reactions to western red cedar (Thuja plicata). CMAJ. 1971;105: 56-61.
4. Manso L, Heili S, Fernández-Nieto M, Sastre B, Sastre J. Basophil activation in two cases of hydrochlorothiazide-induced noncardiogenic pulmonary edema. Allergy. 2010;65:135-6.
5. Frew A, Chan H, Dryden P, Salari H, Lam S, Chan-Yeung M. Immunologic studies of the mechanisms of occupational asthma caused by western red cedar. J Allergy Clin Immunol. 1993;92:466-78.

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Frey Syndrome in Children: A Nonallergic Cause of Facial Erythema Triggered By Food

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Key words: Food allergy. Auriculotemporal nerve syndrome. Frey syndrome in children. Facial erythema. Forceps.

Palabras clave: Alergia alimentaria. Síndrome del nervio auriculotemporal. Síndrome de Frey. Eritema facial. Fórceps.

Food allergy is frequently overdiagnosed, particularly in individuals who develop a rash immediately after ingestion. This may lead to unnecessary tests and delayed diagnosis.

Frey syndrome, otherwise known as auriculotemporal syndrome or gustatory flushing, is relatively common in adults following nerve injury in parotid surgery but it has also been reported in children. It is relatively unknown among primary care providers and is usually confused with an underlying food allergy. We report a series of cases of Frey syndrome in children and emphasize several aspects that should lead any medical practitioner to reject a diagnosis of food allergy.

Nine children referred to our outpatient clinic in the last 10 years presented linear flushing in the auriculotemporal region that normally appeared during the intake of solid or acidic foods. The studies to exclude food allergy consisted of a complete description of clinical symptoms, skin tests (prick and/or prick to prick) and/or oral challenges with the implicated foods.

The children, 6 boys and 3 girls, were aged between 5 months and 7 years. Only 1 of them had a previous diagnosis of atopic disease (dermatitis, asthma, or food allergy). Gestational age at birth was at least 38 weeks, but delivery in all cases had been complicated by failure to progress and required the use of forceps. During their first years of life, the children developed transient flushing on the cheek after eating several foods. The distribution of the erythema was unilateral in most cases and always in the same location, ie, in a line between the edge of the mouth and the ears. Only 1 of the patients had a bilateral distribution.

None of the children had associated symptoms such as swelling, pruritus, pain, discomfort, redness of the eyes, or systemic reactions. Erythema appeared more predictably during the ingestion of fruit, tomato sauces, meat, snacks, candies, or acidic foods. The flushing appeared during chewing and disappeared within minutes.

Physical examination showed no swelling, tenderness of the face, or lymphadenopathy, and the parotid gland region was normal in all cases.

Skin prick tests to suspected foods were negative in all cases. Oral food challenges were performed in 4 children and triggered the almost immediate appearance of the characteristic linear erythema. The flushing lasted for 5 to 30 minutes and there were no other symptoms. The children were all diagnosed with Frey syndrome and did not require treatment. The clinical features and oral food challenge results are shown in the Table.

In routine clinical practice, a number of adverse reactions to foods may be misinterpreted as allergic reactions. We have described 9 patients who attended our outpatient clinic for a food allergy study and were diagnosed with Frey or auriculotemporal syndrome or gustatory flushing. The condition was first described by Duphenix in 1757, but it was rediscovered by Frey [1] in 1923. It is characterized by recurrent episodes of facial gustatory flushing and sweating (more frequent in adults) limited to the cutaneous distribution of the auriculotemporal nerve during the chewing of various types of food. No other symptoms are observed. The syndrome has been reported in children as a sequel of perinatal birth trauma of the auriculotemporal nerve resulting from forceps-assisted delivery. When injured, the nerve undergoes abnormal regeneration. The aberrant parasympathetic nerves fibers are stimulated during the chewing of diverse foods, resulting in pathological vasodilatation rather than salivation [2].

In the case of adults, recent publications have reported the onset of this disorder in patients who have undergone parotid surgery [3,4]; the disorder commonly appears within 1 month to 5 years of surgery and affects 37% to 100% of such patients [5].

Frey syndrome is relatively uncommon in children and is usually related to perinatal birth trauma [6], although congenital nerve injury has also been postulated as a potential cause [7]. There have also been recent reports of an association with facial plexiform neurofibromas [8] and congenital hemangiopericytoma [9].

All of the 9 children in our series had been delivered with the assistance of forceps and none of them had a relevant history of infection, surgery, or accidental trauma. Only 1 of the 9 children had clinical hyperhidrosis. While this is the predominant symptom in adults, it is unusual in children. A diagnosis of food allergy was initially considered by the pediatrician in all of the cases reported, with lengthy investigations, causing anxiety among some parents, before Frey syndrome was diagnosed.

Although a diagnosis of Frey syndrome can be based exclusively on reported symptoms, it is usually diagnosed several months and sometimes even several years after the onset of symptoms [10]. It is noteworthy that in our series, some of the children had been avoiding certain foods for more than 4 years because they had not been diagnosed correctly. Misdiagnosis can generate considerable costs and unnecessary tests and elimination diets, all of which can be easily avoided by increasing awareness of this syndrome.

Several aspects should be considered by physicians to discard food allergy; these include the presence of unilateral flushing always in the same location, the absence of other symptoms (eg, pruritus, vomiting, diarrhea), rapid resolution, and the diversity of foods—mostly acidic—that cause symptoms.

Frey syndrome in children is self-limiting, benign, and does not require treatment. Familiarity with this condition among allergists and pediatricians should help to reduce the misinterpretation of this relatively rare condition as a food allergy.

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Table. Clinical Features and Oral Food Challenge Results in Frey Syndrome

Patient	1	2	3	4	5	6	7	8	9
Past history	Obstetrical brachial plexus lesion	Egg and cow's milk allergy	NR	NR	NR	NR	NR	NR	NR
Age at onset, mo	12	34	5	18	6	6	5	5	6
Age at diagnosis, mo/y	20 mo	7 y	6 mo	24 mo	7 mo	14 mo	5 y	5 mo	3 y
Food involved	Egg Chicken Pork Hake	Candies Fries Burger	Apple Orange Banana Pear	Bread Egg Candies Kiwi Watermelon Strawberry Kiwi	Apple Banana Pear Lemon Tomato Celery Onion Pea Corn Candies	Orange Cookies	Kiwi Pineapple Grapes Lemon Apple Orange Peach Fries Fried tomato Candies	Apple Banana Pear	Candies Fries Fried Chicken
Symptoms	Erythema on the right cheek	Erythema on the right cheek	Erythema on the left cheek	Erythema and sweating on the left cheek	Erythema the left cheek	Erythema the left cheek	Erythema on the right cheek	Erythema on both cheeks	Erythema on the right cheek
Oral food challenge	ND	Candies	ND	Bread	Banana	ND	Fries Candies	ND	ND

Abbreviations: ND, not done; NR, not relevant.

References

1. Frey L. Le syndrome du nerf auriculo-temporal. Rev neurol. 1923; 2:97.
2. Escudero-Canto MC, Cuartero-del P, I, Ruiz-Cano R, Balmaseda-Serrano E, Gil- delivery. Auriculotemporal nerve syndrome in children secondary to a forceps delivery. Rev Neurol. 2007; 44:186.
3. Elliott RM, Weinstein GS, Low DW, Wu LC: Reconstruction of Complex Total Parotidectomy Defects Using the Free Anterolateral Thigh Flap: A Classification System and Algorithm. Ann Plast Surg. 2011 May;66(5):429-37.
4. Singh N, Kohli M, Kohli H. Innovative Technique to Reduce Incidence of Frey's Syndrome after Parotid Surgery. Am Surg. 2011;77:351-4.
5. Thoma-Uszynski S, Mahler V. Incomplete auriculotemporal nerve syndrome--mimicry of oral allergy syndrome. Eur J Dermatol. 2007;17:157-9.
6. Martínez-Baylach J, Aragó T, Galdós H, Herrera C, Rubio de Abajo I. Frey's sydrome secondary to an obstetrics trauma: Presentation of 2 cases and a review of the literature. An Pediatr (Barc). 2010 Apr;72(4):272-7.
7. Sethuraman G, Mancini AJ. Familial auriculotemporal nerve (Frey) syndrome. Pediatr Dermatol. 2009 May-Jun;26(3):302-5.
8. Listernick R, Legius E, Charrow J. Gustatory flushing (auriculotemporal nerve syndrome) in children with neurofibromatosis type 1 and facial plexiform neurofibromas. J Pediatr. 2011 Jun;158(6):1034-34
9. Farman M, Zaitoun H. Auriculotemporal nerve syndrome in association with congenital haemangiopericytoma: a case report. Eur J Paediatr Dent. 2010 Dec;11(4):213-5.
10. Sampson HA. Differential diagnosis in adverse reactions to foods. J Allergy Clin Immunol. 1986;78:212-219.

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Anaphylaxis Due to Orange Soft Drinks

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Key words: Orange. Soft drinks. Anaphylaxis. Cit s 1.

Palabras clave: Naranja. Refresco de naranja. Anafilaxia. Cit s 1.

Citrus sinensis is a tree that belongs to the Rutaceae family; its fruit, the sweet orange, is widely consumed throughout Europe. Valencia, which is the largest producer of sweet oranges in Spain, is one of the most important orange-growing areas in the world. Spain alone produced 5.7 million tons of this fruit in 2011. Sweet orange is not considered to be a common allergenic fruit, although 3 allergens have been described to date: Cit s 1 (germin-like protein), Cit s 2 (profilin), and Cit s 3 (nonspecfic lipid transfer protein) [1,2]; this last allergen has been found to show cross-reactivity with the major peach allergen Pru p 3 [3]. However, no cases of allergy to orange soft drinks have been reported in the literature.

We report the case of a 23-year-old woman who experienced an episode of anaphylaxis 10 minutes after handling sweet oranges in a fruit warehouse (an orange storage network), where she had been working for a month. Because of this episode, she left the job and some months later experienced contact urticaria when squeezing an orange; she also experienced oral allergy syndrome after drinking several orange soft drinks (Sunny Delight and TriNa orange) that she had previously tolerated.

Surprisingly, despite living in Valencia, she had not eaten sweet oranges for 15 fifteen years as she did not like them. She denied having clinically allergic rhinitis or asthma and tolerated various types of fruits, including citric fruit such as lemon, which she tolerated in ice cream and juice form before and after the allergic episodes described above.

Skin prick tests (SPTs) were negative for mites, fungi, and pollen (*Parietaria judaica*, *Olea europaea*, Gramineae family, *Cupressus arizonica*, *Platanus acerifolia*, and *Artemisia vulgaris*) and a prick test with profilin from *Gramineae* pollen was also negative. Extracts from sweet orange pulp and peel were prepared as follows. The pulp and peel were ground into small pieces, defatted, and extracted by magnetic stirring in 50 mM of phosphate-buffered saline (PBS) at pH 7.5 for 3 hours at room temperature. The samples were centrifuged at 5600×g for 30 minutes, and the supernatants dialyzed against water and freeze-dried. The orange soft drink extracts (Sunny Delight and TriNa) were prepared by dilution in phosphate buffer (1:2), followed by magnetic stirring for 15 minutes at room temperature, dialysis, and freeze-drying. Positive SPT

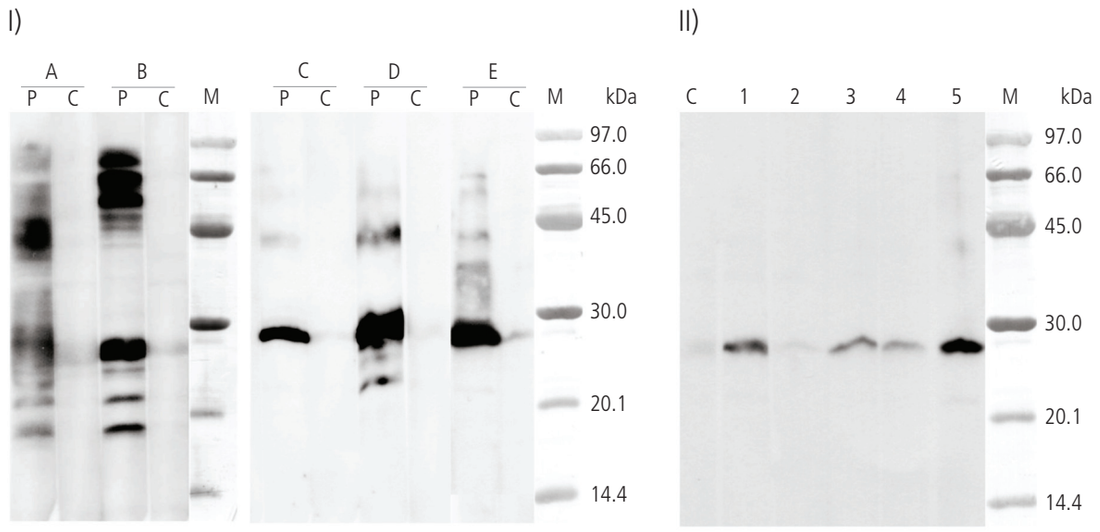

Figure. I) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) immunoblotting results under reducing conditions. A: orange peel extract; B: orange pulp extract; C: Sunny Delight soft drink extract; D: TriNa soft drink extract; E: KAS soft drink extract. Lane P: patient serum (dilution 1:1); lane C: control serum (pool of sera from nonatopic individuals); lane M: molecular mass markers. II) SDS-PAGE immunoblotting-inhibition results with Sunny Delight extract in solid phase. Lane C: control serum (pool of sera from nonatopic individuals); lane 1: patient serum previously incubated with Sunny Delight extract; lane 2: patient serum previously incubated with orange pulp extract; lane 3: patient serum previously incubated with TriNa extract; lane 4: patient serum previously incubated with KAS extract; lane 5: patient serum previously incubated with lamb extract; lane M: molecular mass markers.

results were obtained for the pulp extract (4 × 4 mm) and the peel extract (44 × 5 mm); the results for similar tests with lemon pulp and peel extracts (all at 10 mg/mL) were all negative. The SPTs with the soft drink extracts were negative but the corresponding intradermal tests were positive in both cases (>64 × 6 mm). Furthermore, 1 hour after the intradermal test with orange extract (pulp and peel), the patient developed facial edema, dyspnea, and wheezing that required urgent medical attention.

Total serum immunoglobulin (Ig) E (UniCAP, Phadia) was 6.7 IU/mL and serum specific IgE by means of an enzyme allergosorbent test against orange peel and pulp extracts was <0.35 kU/L (class 0).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) immunoblotting was performed with sweet orange pulp extract. IgE immunoblotting with the patient's serum revealed IgE-binding bands of approximately 75 kDa, 66 kDa, 57 kDa, 28 kDa, 22 kDa, and 18 kDa. The immunoblotting profile of 3 orange soft drink extracts (Sunny Delight, TriNa, and KAS) showed a strong band of 28 kDa. SDS-PAGE immunoblotting-inhibition assays showed the capacity of the orange pulp extract to inhibit the IgE binding to the Sunny Delight extract; partial inhibition was observed with the extracts from the 3 orange refreshments (Figure). This difference in IgE binding–inhibition activity must be due to the higher concentration of Cit s 1 in the orange pulp sample than in the soft drink samples.

The 28-kDa IgE binding protein was identified by MALDI-TOF (matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry) and LC-ESI-MS/MS (liquid chromatography electrospray ionization tandem mass spectrometry), and by searching the NCBI non-redundant protein sequence database using the Mascot program (http://www.matrixscience.com), as previously described [4]. The resulting peptides analyzed by MS or MS/MS corresponded to Cit s 1.

To clarify the implication of this allergen, we suggested performing an oral provocation test with orange, but this was rejected by the patient due to the symptoms she had developed after the intradermal test.

Ahrazem et al [3] showed a high frequency of Cit s 1 sensitization in individuals who did not develop symptoms on consumping oranges; this was attributed to the monovalent nature of this protein based on its N-glycan epitope. For this reason, Cit s 1 has been labeled an "equivocal allergen" [5].

We have presented an interesting case of a nonatopic patient who showed clinically relevant monosensitized allergy to Cit s 1, a sweet orange allergen, which appeared as an active allergenic component in orange soft drinks. Our findings are consistent with the IgE reactivity exhibited by Cit s 1 in heat-processed orange juice described by Crespo et al [1].

To conclude, we believe that the clinical relevance of Cit s 1 should be studied more carefully, with an independent investigation of each case.

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References

1. Crespo JF, Retzek M, Foetisch K, Sierra-Maestro E, Cid-Sanchez AB, Pascual CY, Conti A, Feliu A, Rodriguez J, Vieths S, Scheurer S. Germin-like protein Cit s 1 and profilin Cit s 2 are major allergens in orange (Citrus sinensis) fruits. Mol Nutr Food Res. 2006 Mar;50(3):282-90.
2. López-Torrejón G, Ibáñez MD, Ahrazem O, Sánchez-Monge R, Sastre J, Lombardero M, Barber D, Salcedo G. Isolation, cloning and allergenic reactivity of natural profilin Cit s 2, a major orange allergen. Allergy. 2005 Nov;60(11):1424-9.
3. Ahrazem O, Ibáñez MD, López-Torrejón G, Sánchez-Monge R, Sastre J, Lombardero M, Barber D, Salcedo G. Orange germin-like glycoprotein Cit s 1: an equivocal allergen. Int Arch Allergy Immunol. 2006;139(2):96-103.
4. Pastor C, Cuesta-Herranz J, Cases B, Pérez-Gordo M, Figueredo E, de las Heras M, Vivanco F. Identification of major allergens in watermelon. Int Arch Allergy Immunol. 2009;149(4):291-8.
5. Pöltl G, Ahrazem O, Paschinger K, Ibañez MD, Salcedo G, Wilson IB. Molecular and immunological characterization of the glycosylated orange allergen Cit s 1. Glycobiology. 2007;Feb;17(2):220-30.

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Nasal Challenge Test in the Diagnosis of Latex-Related Systemic Reactions

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Key words: Anaphylaxis. Nasal challenge test. Natural rubber latex.

Palabras clave: Anafilaxia. Prueba de provocación nasal. Látex.

Natural rubber latex (NRL) allergy is recognized as a major health problem. Around 12% of allergic reactions occurring during anesthesia are caused by latex [1], although the incidence of latex allergy seems to have decreased in recent years [2]. None of the available diagnostic tests has 100% sensitivity.

We report the case of a 30-year-old woman who experienced anaphylactic shock during a myomectomy under general anesthesia (serum tryptase, 18.6 ng/mL at 1 hour after the reaction). The patient had a personal history of mild rhinitis and house dust mite sensitization, but neither drug-related nor food-related allergic reactions had been reported. She worked as a dental assistant and had never experienced problems with NRL gloves or occupational respiratory symptoms.

In vivo study: The results of skin prick tests (SPT) and intradermal tests with the drugs used during anesthesia (propofol, fentanyl, midazolam, cisatracurium, and cefminox) were all negative. We performed an intramuscular challenge test with cefminox, although no allergic reaction was observed. SPT using commercial latex extract (100 IR/mL, Stallergènes) was not conclusive (wheal 3 × 3 mm without erythema). Therefore, to confirm the suspected NRL allergy, we performed a prick-by-prick test with a latex glove, a rubbing test, and a glove use test, although the results were negative in all cases. In order to rule out a possible lack of efficacy of the first commercial latex extract, SPT was repeated using another commercial extract with a known latex protein concentration of 0.5 mg/mL (ALK-Abelló). We tested several dilutions (1/100 weight/volume [w/v], 1/10 w/v, and 1/1 w/v), although only the undiluted SPT (0.5 mg/mL) was slightly positive, with a wheal of 4 × 4 mm (histamine, 9 × 8 mm). We mimicked mucosal allergen exposure by performing a nasal challenge test (NCT) with the NRL extract used in the second SPT. The challenge was performed according to guidelines [3] and monitored using acoustic rhinometry. Although SPT had only been positive at 1/1 w/v, NCT was performed at 2 different concentrations (0.5 mg/mL and 0.05 mg/mL). The result was positive (decrease of >25% in nasal volume between 0 and 7 cm) only at 0.5 mg/mL. No systemic reactions were observed. A healthy control was also tested after providing written informed consent, with negative results. A thorough investigation into food sensitization related to NRL allergy (banana, kiwi, avocado, and chestnut) was negative (both history and SPT).

In vitro study: Specific immunoglobulin (Ig) E to NRL

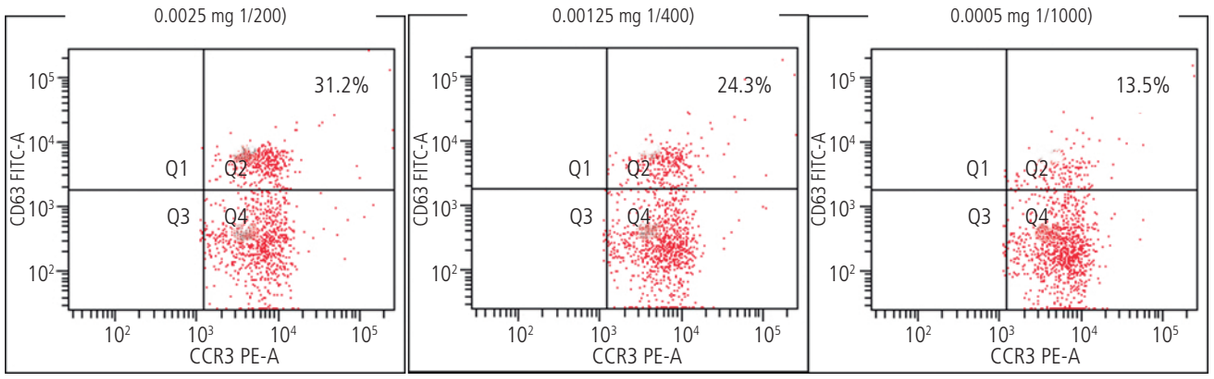

Figure. Basophil activation test. The stimulus used was natural rubber latex in 0.9% NaCl solution (0.5 mg/mL) at 3 dilutions (1:200, 1:400, and 1:1000). All concentrations gave a positive result.

was 0.33 kU_A/L (specific IgE level >0.35 kU_A/L was considered positive) and negligible to banana, kiwi, avocado, and chestnut extracts (ImmunoCAP, Phadia). Baseline tryptase was 4.02 ng/mL (ImmunoCAP). The patient's serum was also tested against 103 allergenic components in a commercially available microarray (ImmunoCAP ISAC, Phadia) including the major allergenic components of latex (Hev b 1, Hev b 3, Hev b 5, and Hev b 6) and the cross-reactivity marker (Hev b 8). Specific IgE was undetectable (specific IgE >0.3 ISAC standardized units was considered positive). Given the high clinical suspicion, specific IgE determination was repeated using the Advia-Centaur IgE assay (ALK-Abelló), which showed that specific IgE for the allergenic components of latex was positive for Hev b 6 (1.75 kU_A/L) and negative for Hev b 1, Hev b 8, and Hev b 5. A basophil activation test was also performed on fresh whole blood using the Flow2CAST kit (Bühlmann), following the manufacturer's protocol. The stimulus used was the same preparation of NRL in 0.9% saline solution (0.5 mg/mL) used for NCT, but at various dilutions in phosphate-buffered saline (1:200, 1:400, and 1:1000), as reported elsewhere [4]. All concentrations gave a positive test result for the patient (Figure) and negative result for a nonatopic control tested in parallel.

We describe an episode of anaphylaxis during anesthesia that was probably due to an IgE-mediated hypersensitivity reaction to NRL. A potential role of the drugs used during surgery was ruled out by negative results in a challenge test with cefminox and the high sensitivity of skin tests with general anesthetics [5,6]. Although our patient was mainly sensitized to prohevein (Hev b 6), a major allergenic component of latex, most in vivo tests were not conclusive in her case, except for NCT. The importance of NCT in NRL allergy diagnosis has been already reported [7,8]. Üntel et al [7] demonstrated very high sensitivity (96%) and specificity (100%) for NCT with latex in patients experiencing respiratory symptoms with NRL (higher than those obtained in the glove use test [81% sensitivity and 90% specificity]), with a very good safety profile. Palczynski et al [8] studied 16 nurses with a positive NCT result to latex and NRL-associated asthma or rhinitis; they all had positive SPT but negative specific IgE. The basophil activation test is considered a highly reliable in vitro procedure for the detection of IgE-mediated allergy to latex, given its high sensitivity (95%) and specificity (100%) [4].

To our knowledge, this is the first report of perianesthetic latex-related anaphylaxis to be confirmed in vivo using NCT only. This test seems to be a simple, useful, and safe tool for the diagnosis of latex allergy beyond respiratory symptoms. Therefore, it should be taken into account when latex allergy is suspected and conventional diagnostic tools (SPT and serum specific IgE) are not conclusive or other in vitro tests are not available. As latex can trigger potentially severe reactions, all in vivo latex allergy tests, NCT included, should be performed in the hospital by well-trained medical staff with resuscitation facilities on hand in case of emergency.

References

1. Mertes PM, Laxenaire MC. Allergic reactions occurring during anaesthesia. Eur J Anaesthesiol. 2002;19:240-62.
2. Mertes PM, Laxenaire MC; GERAP. Anaphylactic and anaphylactoid reactions occurring during anaesthesia in France. Seventh epidemiologic survey (January 2001-December 2002). Ann Fr Anesth Reanim. 2004;23:1127-8.
3. Litvyakova LI, Baraniuk JN. Nasal provocation testing: a review. Ann Allergy Asthma Immunol. 2001;86:355-65.
4. Nettis E, Colanardi MC, Pia Dambra M, Loria MP, Ferrannini A, Vacca A Tursi A. Flow cytometric basophil activation test: detection of CD63 expression as a useful aid to diagnosis of latex allergy. Ann Allergy Asthma Immunol. 2006;97:715-6.
5. Moneret-Vautrin DA. Tests cutanés pour le diagnostic d'allergie aux curares. Ann Fr Anesth Réanim. 2002;21:97-107.
6. Laxenaire MC, Mata-Bermejo E, Moneret-Vautrin DA, Gueant JL. Life-threatening anaphylactoid reactions to propofol (Diprivan) Anesthesiology. 1992;77:275-80.
7. Ünsel M, Mete N, Ardeniz O, Göksel S, Ersoy R, Sin A, Gulbahar O, Kokuludag A. The importance of nasal provocation test in the diagnosis of natural rubber latex allergy. Allergy. 2009;64:862-7.
8. Palczynski C, Walusiak J, Ruta U, Gorski P. Nasal provocation test in the diagnosis of natural rubber latex allergy. Allergy. 2000;55:34-41.

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Persistence of Allergy to Goat's Milk After Specific Induction of Tolerance to Cow's Milk

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Key words: Cow's milk allergy. Food allergy. Goat's milk allergy. Oral desensitization. Specific oral tolerance induction.

Palabras clave: Alergia a leche de vaca. Alergia alimentaria. Alergia a leche de cabra. Desensibilización oral. Inducción de tolerancia oral específica.

Cow's milk protein (CMP) allergy is one of the most common causes of food allergy in childhood. In Spain, the incidence of allergy to cow's milk in the first year of life ranges between 0.36% and 1.9% [1,2]. Most allergic children achieve tolerance during the first 4 years of life, although the disease has been reported in children as old as 16 years [3].

Caseins are major allergens in CMP allergy, and a high degree of cross-reactivity has been demonstrated between caseins in the milk of different species of animals. Cross-reactivity results from homology between the amino acid sequences of these proteins [4].

Children who are allergic to CMPs are sensitized to whey proteins, the casein fraction, or both, and many also react to goat's milk or sheep's milk because of the high degree of homology between the corresponding proteins. Conversely, goat's milk allergy does not usually involve allergic cross-reactivity to cow's milk. The allergens responsible are mainly caseins and calcium-binding proteins, such as αS1-casein, αS2-casein, and ß-casein, but not whey proteins [5].

Treatment of CMP allergy is based on avoidance of cow's milk and its derivatives, as well as milk and dairy products from other mammals (especially goat and sheep), because of cross-reactivity between different species. The most recent treatment option is specific oral tolerance induction (SOTI). However, it is important to note that SOTI to CMPs does not induce tolerance to milk from other mammals, as demonstrated in a recent study [6].

The aim of this study was to determine differences in the pattern of sensitization to goat's milk proteins in goat's milk–tolerant and cow's milk–intolerant patients who successfully completed SOTI to cow's milk.

We report 8 children sensitized to goat's milk with good tolerance to cow's milk after SOTI. The sample was divided into 2 groups: group A included 4 children (aged 2 to 9 years) who were desensitized to CMP at age 2 to 6 years but who did not tolerate goat's milk (goat's milk immunoglobulin [Ig] E, 12.5-100 kU_A/L); group B included 4 children (aged 3 to 8 years) who were desensitized to CMP at age 2 to 5 years but who tolerated goat's milk (goat's milk IgE, 0.9-40.6 kU_A/L).

Skin prick tests were performed with goat's milk (10 mg/mL) (DIATER), whole cow's milk (10 mg/mL) (ALK-Abelló), and isolated CMPs: casein (10 mg/mL) (LETI), α-lactalbumin (5 mg/mL), and ß-lactoglobulin (5 mg/mL) (DIATER) following standardized methodology [7].

Serum determinations of specific IgE to goat's milk, cow's milk, and isolated CMPs were performed using CAP-FEIA (Phadia).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was carried out using cow's milk and goat's milk following the methodology of Towbin et al [8]. Immunoblotting inhibition was carried out with serum pools from each group incubated with serial dilutions of cow's milk and goat's milk (protein content of 10, 1, and 0.1 mg).

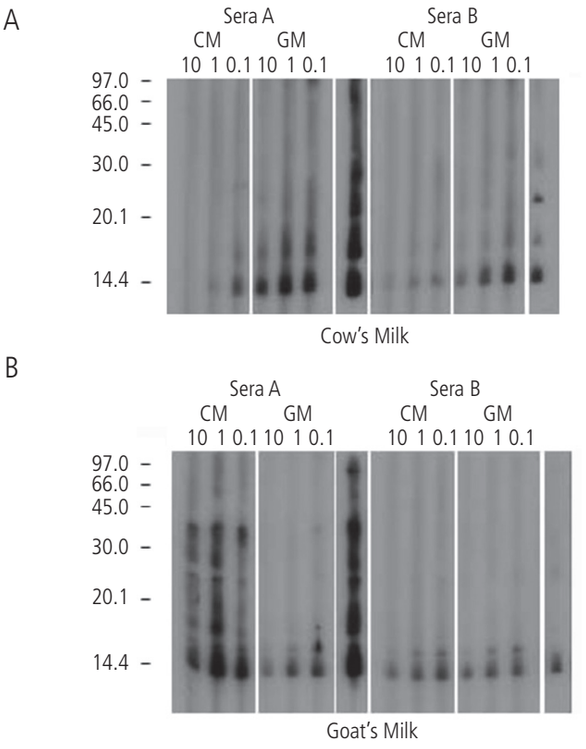

Figure. Immunoblotting of immunoglobulin E against cow's milk (A) and goat's milk proteins (B) inhibited with cow's milk (CM) and goat's milk (GM).

Open controlled challenge tests with goat's milk were performed in the Allergy Unit of our hospital (Hospital General Universitario, Valencia, Spain), where appropriate medication and resuscitation equipment were available. Informed consent was previously obtained from the parents. All challenge tests were carried out under the control of an allergist.

Patients in group A had multiple IgE binding bands for CMPs and goat's milk proteins (molecular weight range compatible with casein, α-lactalbumin, and ß-lactoglobulin). Sera from patients in group B recognized multiple bands for CMPs, although at low intensity and only in the range of the α-caseins and ß-caseins of goat's milk proteins. Sera from patients in group B were completely inhibited with cow's milk. Sera from patients in group A reacted with goat's milk proteins in the range of caseins after inhibition with cow's milk (Figure).

Cow's milk SOTI has proven to be an effective therapeutic procedure that achieves tolerance in most CMP-allergic patients [9]. Nevertheless, some patients can present allergic reactions after ingestion of goat's milk [6].

The cross-reaction between CMPs and goat's milk proteins means that most patients who are allergic to cow's milk cannot tolerate goat's milk [10]. Conversely, goat's milk allergy does not usually involve an allergic reaction to cow's milk. A patient can become sensitized to specific proteins of goat's milk without

experiencing cross-reactions with CMPs, thus explaining why allergic patients tolerate cow's milk, but not goat's milk [5].

The results of this study suggest that patients who do not tolerate goat's milk after cow's milk SOTI are sensitized to specific goat's milk caseins without cross-reactivity to CMPs. Cow's milk SOTI can induce tolerance to CMPs but does not guarantee tolerance to the milk of other mammals. Once cow's milk SOTI has been completed, a diet free of goat's milk should be adhered to until tolerance has been tested using controlled challenge testing.

We conclude that patients who are allergic to goat's milk after cow's milk SOTI show sensitization to goat's milk proteins that have no cross-reactivity with CMPs, thus explaining the persistence of clinical reactivity and confirming the specificity of SOTI.

References

1. Sanz Ortega J, Martorell A, Michavila A, Nieto A y grupo de trabajo para alergia alimentaria. Estudio de la incidencia mediada por IgE frente a la proteína de leche de vaca en el primer año de vida. Ann Esp Pediatr. 2001;53:536-9.
2. García Ara MC, Boyano MT, Díaz Pena JM, Martín Muñoz F, Pascual C, García Sánchez G, Martín Esteban M. Incidencia de alergia a leche de vaca y su repercusión en el consumo de hidrolizados. An Pediatr. 2003;58:100-5.
3. Skripak JM, Matsui EC, Mudd K, Wood RA. The natural history of IgE-mediated cow's milk allergy. J Allergy Clin Immunol. 2007;120:1172-7.
4. Restani P, Gaiaschi A, Plebani A, Beretta B, Cavagni G, Fiocchi A, Poiesi C, Velonà T, Ugazio AG, Galli CL. Cross-reactivity between milk proteins from different animal species. Clin Exp Allergy. 1999;29:997-1004.
5. Ah-Leung S, Bernard H, Bidat E, Paty E, Rancé F, Scheinmann P, Wal JM. Allergy to goat and sheep milk without allergy to cow's milk. Allergy. 2006;61:1358-65.
6. Alonso-Lebrero E, Fuentes V, Zapatero L, Pérez-Bustamante S, Pineda F, Martinez-Molero MI. Goat's milk allergies in children following specific oral tolerance induction to cow's milk. Allergol Immunopathol. 2008;36:180-1.
7. Dreborg S, Frew A. Position paper allergen standardization and skin tests. Allergy. 1993;47 (Suppl. 14):48-82.
8. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA. 1979;76:4350-4.
9. Martorell A, De la Hoz B, Ibañez MD, Bone J, Terrados MS, Michavila A Plaza AM, Alonso E, Garde J, Nevot S, Echeverria L, Santana C, Cerdá JC, Escudero C, Guallar I, Piquer M, Zapatero L, Ferré L, Bracamonte T, Félix R, Martínez MI. Oral desensitization as a useful treatment in 2-year-old children with cow's milk allergy. Clin Exp Allergy. 2011;41:1297-1304.
10. Bellioni-Businco B, Paganelli R, Lucenti P, Giampietro PG, Perborn H, Businco L. Allergenicity of goat's milk in children with cow's milk allergy. J Allergy Clin Immunol. 1999;103:1191-4.

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Successful Desensitization to Rabbit Antithymocyte Globulin in a Patient With Aplastic Anemia

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Key words: Aplastic anemia. Antithymocyte globulin. Drug reaction. Anaphylaxis. Desensitization.

Palabras clave: Anemia aplásica. Globulina antitimocito. Reacción a drogas. Anafilaxia. Desensibilización.

Aplastic anemia is a type of peripheral blood pancytopenia in which hypocellular bone marrow (<25% cellularity) is confirmed by biopsy. The choice between the 2 possible treatment modalities—bone marrow transplantation and immunosuppression—depends on the availability of a histocompatible family donor, patient age, and severity of the aplasia. Immunosuppressive therapy includes antithymocyte globulin (ATG), ciclosporin, prednisone, and other drugs [1].

A 15-year-old girl with no history of allergy was referred to the hematology department of our hospital with thrombocytopenia that had been detected in June 2010. Blood tests revealed pancytopenia in peripheral blood. Bone marrow biopsy showed marked hypoplasia of the 3 hematopoietic series (<25%). She was finally diagnosed with idiopathic aplastic anemia. Bone marrow transplant from a histocompatible sibling was proposed, although this was not possible because the potential donor was diagnosed with mixed connective tissue disease. Therefore, immunosuppressive therapy with rabbit ATG (rATG), ciclosporin A, and prednisone was prescribed. As recommended [2], 1 hour before the infusion of rATG, the patient was premedicated with acetaminophen 1 g, methylprednisolone 75 mg, and dexchlorpheniramine maleate 5 mg. rATG therapy was started at 25 mg/h (total scheduled infusion of 6 hours). After 90 minutes the patient developed pharyngeal itching and dyspnea. The infusion was stopped, hydrocortisone 100 mg was administered, and her condition improved within 20 minutes. Five hours later, the infusion was restarted more slowly to be administered over 12 hours; however, after 90 minutes the patient complained of pharyngeal itching, dyspnea, chest tightness, wheezing, and generalized hives. Her condition improved after administration of intravenous hydrocortisone. Treatment with rATG was discontinued, and the drug allergy unit of our department was consulted to assess desensitization to rATG.

Skin tests were performed following the recommendations of the European Academy of Allergy and Clinical Immunology [3]. Prick testing was performed with a commercial extract of rabbit epithelium (ALK-Abelló). Serum tryptase and total and specific immunoglobulin (Ig) E were determined using ImmunoCAP (Phadia). Prick testing with undiluted rATG at 5 mg/mL (Genzyme Corporation) and intradermal testing with 0.05 mL diluted in saline from 1:100 000 to 1:100 were performed. Skin testing

Table. Incidents During Desensitization to Rabbit Antithymocyte Globulin

Day 1	Day 2	Day 3	Day 4	Day 5
8:30 AM Start of infusion	12:10 PM Extensive erythema with severe itching	8:00 AM Slightly pruriginous erythema on trunk, arms, and neck	Mild nonpruritic erythema	No symptoms Phlebitis on right arm (intravenous line)
2:00 PM Mild nonpruritic erythema on trunk (15 minutes' duration)	3:00 PM Itching on scalp and arms with erythema on neck and arms that lasted 1 hour; chest tightness without dyspnea or wheezing (1 hour's duration) 1 episode of vomiting			
	7:00 PM Diarrhea			
	9:00 PM Erythema on legs that lasted 1 hour			
Normal vital signs	Normal vital signs	Normal vital signs	Normal vital signs	Normal vital signs
No treatment	Intravenous dexchlorpheniramine maleate 5 mg	Prescription of oral dexchlorpheniramine maleate 6 mg at midday	No treatment	No treatment

was not performed in healthy controls for ethical reasons (risk of sensitization to rATG). After obtaining informed consent, we applied a previously described protocol for rapid intravenous desensitization to chemotherapy and monoclonal antibodies. We modified the protocol in order to finish in 24 hours instead of the previously described 5.8 hours [4]. Three solutions of rATG (1.5, 15, and 150 mg) in saline (250 mL) were delivered in 12 consecutive steps at increasing rates every 15 minutes. From step 12, the infusion rate remained unchanged over 24 hours for 5 days (150 mg/d). Dexchlorpheniramine maleate 5 mg and methylprednisolone 75 mg were administered intravenously every 12 hours.

The results of prick testing with rabbit epithelium and undiluted rATG and specific IgE to rabbit epithelium and meat were negative. Levels of total IgE and serum tryptase were 134 IU/mL and 4.02 μg/L, respectively. Intradermal testing with rATG revealed an increase in the largest diameter of the wheal at 20 minutes, although this was similar at all dilutions (1/100 000 to 1/100).

Drug desensitization was completed successfully, and the patient received rATG for 5 days. The Table summarizes the incidents recorded during the desensitization protocol.

Until 2005, both equine ATG (eATG) and rATG were available. However, the only available preparation since then has been rATG, which is a purified immunoglobulin prepared from hyperimmune serum of rabbits immunized with human thymic lymphocytes. The result is a product rich in antihuman T-cell antibodies, which bind to the surface of circulating T cells and T cells within lymphoid organs, thus reducing the number of functional T lymphocytes and creating an immunosuppressive effect [2,5].

Anaphylaxis has been reported after administration of eATG and rATG [2,5]. Because of the potential for serious allergic reactions, the manufacturer of eATG recommended skin testing prior to treatment, although the predictive value of this approach has not been proven clinically [5]. In contrast, skin testing is not recommended before administering rATG, because it is a poor predictor of anaphylactic reactions [5]. Desensitization protocols have been developed and are used in patients with allergic reactions to various drugs [4].

A search in PubMed revealed only 5 cases of desensitization, all of which were to eATG, but none to rATG. Hall and Hagemann [6] reported a case of successful desensitization to eATG and reviewed 4 previously published cases [7-9], 2 of which had an unsuccessful outcome [9]. Most of these authors [6-9] suspected an IgE-mediated reaction and based their diagnosis on skin testing; however, false-positive results have been recorded with intradermal testing [8].

Although we found intradermal tests to be positive in the immediate reading, we cannot state that the mechanism is IgE-mediated, since no progressive enlargement of the wheal was observed with increasing concentrations of the drug. In addition, a latency period exceeding 1 hour and no prior exposure to rATG rule this mechanism out, and tests would have to be performed on controls to exclude an irritant effect. We suggest other possible mechanisms. Lysis of T lymphocytes is dependent on complement activation [2]; therefore, it is likely that the complement fragment C5a (anaphylatoxin) had a role in the reaction our patient experienced. Moreover, the action of rATG targets multiple CD molecules located on the membrane of T lymphocytes, but which are not exclusive to these cells: some CD molecules are also present in monocytes, basophils, and mast cells [10], with the result that activation of these cells with the subsequent release of their mediators is probable. Finally, lysis of T lymphocytes and monocytes and subsequent release of their cytokines and chemokines, respectively, all of which can act on basophils releasing histamine (histamine-releasing factors), could also explain such a reaction. These complex immunological mechanisms, whether alone or in combination, seem to be a likely explanation for the reaction. Our findings are consistent with those of previously reported reactions to eATG [6-9], namely, symptoms, doubtful interpretation of skin test results, and adverse events that respond to antihistamines during desensitization. These findings strengthen our hypothesis that this type of reaction could be associated with the mechanism of action, which is the same

in both types of ATG, and not with the antigenic characteristics, which are obviously different.

More studies are necessary to clarify the immunological mechanisms involved in these reactions, the most appropriate diagnostic method, and the changes that occur during the desensitization process.

Data from this report were included in a poster presentation at the 30th Congress of the European Academy of Allergy and Clinical Immunology, 11-15 June, Istanbul, Turkey.

References

1. Young NS, Maciejewski JP. Aplastic anemia. In: Hoffman R, Benz EJ, Shattil SS et al., eds. Hematology: Basic Principles and Practice. 5th ed. Philadelphia, PA: Elsevier Churchill Livingstone; 2008. chap 29. p. 359-383
2. Genzyme Corporation. (2009). Thymoglobulin® (anti-thymocyte globulin [rabbit]) [Package insert]. Cambridge, MA: Author.
3. Brockow K, Romano A, Blanca M, Ring J, Pichler W, Demoly P. General considerations for skin test procedure in the diagnosis of drug hypersensitivity. Allergy. 2002;57:45-51.
4. Castells MC, Tennant NM, Sloane DE, Hsu FI, Barrett NA, Hong DI, Laidlaw TM, Legere HJ, Nallamshetty SN, Palis RI, Rao JJ, Berlin ST, Campos SM, Matulonis UA. Hypersensitivity reactions to chemotherapy: outcomes and safety of rapid desensitization in 413 cases. J Allergy Clin Immunol. 2008;122(3):574-80.
5. Bevans MF, Shalabi RA. Management of patients receiving antithymocyte globulin for aplastic anemia and myelodysplastic syndrome. Clin J Oncol Nurs. 2004;8(4):377-82.
6. Hall M, Hagemann TM. Successful desensitization to antithymocyte globulin in a child with aplastic anemia. Am J Health Syst Pharm. 2006;63:1633-6.
7. Ferdman RM, Wakim M, Church JA, Hofstra TC, Thomas D, Genyk YS. Rapid intravenous desensitization to antithymocyte globulin in a patient with aplastic anemia. Transplantation. 2004;77:321-3.
8. Bielory L, Wright R, Nienhuis AW, Young NS, Kaliner MA. Antithymocyte globulin hypersensitivity in bone marrow failure patients. JAMA. 1988;260(21):3164-7.
9. Millar MM, Grammer LC. Case reports of evaluation and desensitization for anti-thymocyte globulin hypersensitivity. Ann Allergy Asthma Immunol. 2000;85(4):311-6.
10. COPE: Horst Ibelgaufts (2011) Cytokines & Cells Online Pathfinder Encyclopaedia at www.copewithcytokines.org.

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Delayed-Type Hypersensitivity to Fenofibrate

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Key words: Lipid-lowering drugs. Fibrates. Cell-mediated drug allergy. Cross-reactivity.

Palabras clave: Medicación hipolipemiante. Fibratos. Alergia a medicamentos mediada por células. Reactividad cruzada.

Statins and fibrates are the 2 most commonly used types of lipid-lowering drugs for the prevention of cardiovascular disease [1]. Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase by reducing endogenous cholesterol synthesis, while fibrates promote catabolism of very-low-density lipoprotein (VLDL) in bile by reducing synthesis of VLDL and blocking HMG-CoA reductase [2].

Statins and fibrates are generally well tolerated. Their side effects are infrequent and include myalgia with consequent increased creatine phosphokinase [3-5] and hepatotoxicity associated with changes in transaminase levels [6-7]. No allergic reactions to these medications have been described.

We report the case of a 77-year-old woman with dyslipidemia who did not tolerate long-term administration of fluvastatin, which was suspended after she experienced myalgia and elevated liver enzyme levels (aspartate and alanine aminotransferase). An alternative lipid-lowering drug, fenofibrate, was prescribed at the normal adult dose (200 mg/d) to control cardiovascular risk. She experienced generalized maculopapular rash immediately after taking the tenth tablet and was treated in the emergency room with oral corticosteroids, whose dose was gradually tapered at home.

The patient was referred to our allergy unit. Her clinical history was negative for other allergic diseases. An allergy workup was performed (skin prick test and patch test with fenofibrate), and an oral challenge test with alternative lipid-lowering agents was planned.

Skin testing was conducted using undiluted solution of the commercial drug obtained from the tablet powder diluted with a drop of saline, as recommended by the European Network on Drug Allergy (ENDA) [8]. We tested the fenofibrate solution on the volar surface of the forearm using the prick method and read the reaction at 20 minutes. A positive control (histamine, 10 mg/mL) and a negative control (0.9% saline) were also used.

Patch testing was performed by applying the undiluted solution in the interscapular region and the reaction was read at 72 hours. An uncoated polyester patch that did not contain any allergen or vehicle was used as a negative control. Positivity was assessed according to the recommendations of the ENDA [8].

We also performed both tests in 5 healthy adults.

The immediate reading of the skin test was negative. The response to the patch test was positive (72 hours), with erythema, wheals, and vesicles at the skin site tested with fenofibrate. The results of the tests in the 5 healthy adults were negative.

The clinical history and the positive patch test results indicated a diagnosis of delayed-type hypersensitivity to fenofibrate.

Considering that the patient needed to use lipid-lowering agents to reduce the risk of heart disease and stroke, we performed oral challenge tests with an ion-exchange resin (cholestyramine) and a dietary supplement (policosanol, red yeast, berberine,

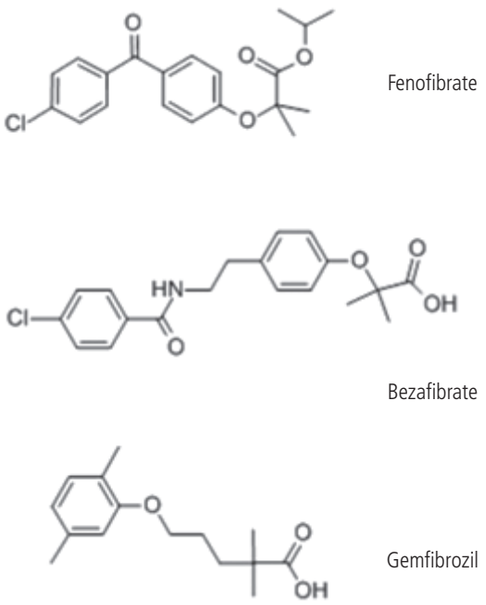

Figure. Chemical structures of fenofibrate, bezafibrate, and gemfibrozil.

folic acid, astaxanthin, and coenzyme Q10). Both agents were well tolerated, although the standard of care required the upper limit for blood cholesterol to remain unchanged. Therefore, we decided to perform a skin prick test and patch test with additional commercialized fibrates (gemfibrozil and bezafibrate). Delayed hypersensitivity was confirmed only for bezafibrate. The cross-reactivity between fenofibrate and bezafibrate may be due to the fact that both medications, unlike gemfibrozil, include 2 benzene rings and the substitution of a chlorine atom in place of the benzoyl-ketone group. The chemical structures of all 3 fibrates are shown in the Figure.

Based on the negative results of the skin prick test and patch test, the patient underwent a single-blind oral challenge test with gemfibrozil (up to 600 mg). As the patient tolerated this fibrate well, she was prescribed the recommended daily dose (600 mg twice a day 30 minutes before the morning and evening meals).

The clinical features and timing of the adverse reaction presented by our patient were highly suggestive of a cell-mediated reaction, although delayed-type reactions to fibrates have not been reported in the medical literature. Patch testing is a useful tool for evaluating nonimmediate reactions to systemic drugs [9].

We show how fibrates, which are generally regarded as safe and well tolerated, can induce an allergic reaction. Our findings are consistent with a diagnosis of cell-mediated allergy to fenofibrate and possible cross-reactivity to bezafibrate.

The changes made in the molecular structure of fenofibrate and bezafibrate to enhance target specificity and therapeutic activity could reasonably explain the lack of cross-reactivity between these fibrates and gemfibrozil. The literature contains no data on the tolerability of gemfibrozil in patients with a delayed-type hypersensitivity reaction to fibrates.

Based on our experience, gemfibrozil could be a safe alternative to fenofibrate. Our findings should be confirmed by further studies with this drug.

References

1. Chyu Ky, Shah PK. Emerging therapies for atherosclerosis prevention and management. Cardiol Clin. 2011;29:123-35.
2. Moutzouri E, Kei A, Elisaf MS, Milionis HJ. Management of dyslipidemias with fibrates, alone and in combination with statins: role of delayed-release fenofibric acid. Vasc Health Risk Manag. 2010;6:525-39.
3. Baker SK, Tarnopolsky MA. Statin-associated neuromyotoxicity. Timely Top Med Cardiovasc Dis. 2005;9:E26.
4. Guis S, Figarella-Branger D, Mattei JP, Nicoli F, Le Fur Y, Kozak-Ribbens G, Pellissier JF, Cozzone PJ, Amabile N, Bendahan D. In vivo and in vitro characterization of skeletal muscle metabolism in patients with statin-induced adverse effects. Arthritis Rheum. 2006;55:551-7.
5. Jamal SM, Eisenberg MJ, Christopoulos S. Rhabdomyolysis associated with hydroxymethylglutaryl-coenzyme A reductase inhibitors. Am Heart J. 2004;147:956-65.
6. Tuteja S, Pyrsopoulos NT, Wolowich WR, Khanmoradi K, Levi DM, Selvaggi G, Weisbaum G, Tzakis AG, Schiff ER. Simvastatin-ezetimibe-induced hepatic failure necessitating liver transplantation. Pharmacotherapy. 2008;28:1188-93.
7. Oms P, Assie N, Bruniguel F, Degryse AD, van Haverbeke G, Delhon A. Biochemical changes and morphological alterations of liver and kidney in hamsters after administration of the HMG-coenzyme A reductase inhibitor, simvastatin: prevention and reversibility by mevalonate. Pharmacol Toxicol. 1995;77:391-6.
8. Brockow K, Romano A, Blanca M, Ring J, Pichler W, Demoly P. General considerations for skin test procedures in the diagnosis of drug hypersensitivity. Allergy. 2002;57:45-51.
9. Lammintausta K, Kortekangas-Savolainen O. The usefulness of skin tests to prove drug hypersensitivity. Br J Dermatol. 2005;152:968-74.

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A Sea Urchin Roe Tropomyosin-Like Protein Is Recognized in Vitro by Shrimp-Allergic Individuals

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Key words: Sea urchin roe. Allergy. Tropomyosin.

Palabras clave: Erizo de mar. Alergia. Tropomiosina.

Shrimp is the most common cause of shellfish allergy. Several allergens have been described, the most allergenic of which is tropomyosin, which in addition is responsible for immunoglobulin (Ig) E cross-reactivity with crustaceans, other arthropods, and mollusks [1]. The safe ingestion of other marine invertebrates by crustacean-allergic patients has not been described, and the general recommendation is to avoid all shellfish, including sea urchins [2]. Sea urchins are marine invertebrates belonging to the phylum echinoderms. They are considered seafood, despite the fact that they are not related to fin fish, mollusks, or crustaceans. Their reproductive organs (roe) are used either raw or briefly cooked in Korean and Japanese cuisine (they are called *uni* in sushi) throughout the world. They are considered a delicacy and are becoming increasingly popular. Although delayed hypersensitivity skin reactions due to stings have been reported [3], immediate hypersensitivity is rare [4-6]. Recently the major allergen of sea urchin roe was identified as a major yolk protein (160 kDa) [7].

This study aimed to address whether shrimp-allergic patients recognize sea urchin allergens. Ten adult and pediatric shrimp-allergic patients with a positive double-blind, placebo-controlled food challenge with shrimp but no previous exposure to urchin roe were selected. IgE recognition of shrimp (*Litopenaeus vannamei*) and urchin roe extracts was evaluated as previously described [8]. Raw and boiled roe extracts were prepared from fresh green sea urchins obtained from a local store. Briefly, the roe was separated from the spiny shell and brown innards and ground. One portion was boiled in distilled water. Protein was extracted from manually homogenized raw and boiled roe by agitation in phosphate buffer saline–containing protease inhibitor cocktail without EDTA (Roche) and with 0.05% sodium azide overnight at 4ºC. The mixture was centrifuged at 3000 rpm for 10 minutes and at 12 600 rpm for 30 minutes at 4ºC. The pellets and supernatants were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Nupage 4-12% Zoom Gels; Invitrogen). The pellets showed the best protein discrimination and thus were used. Protein

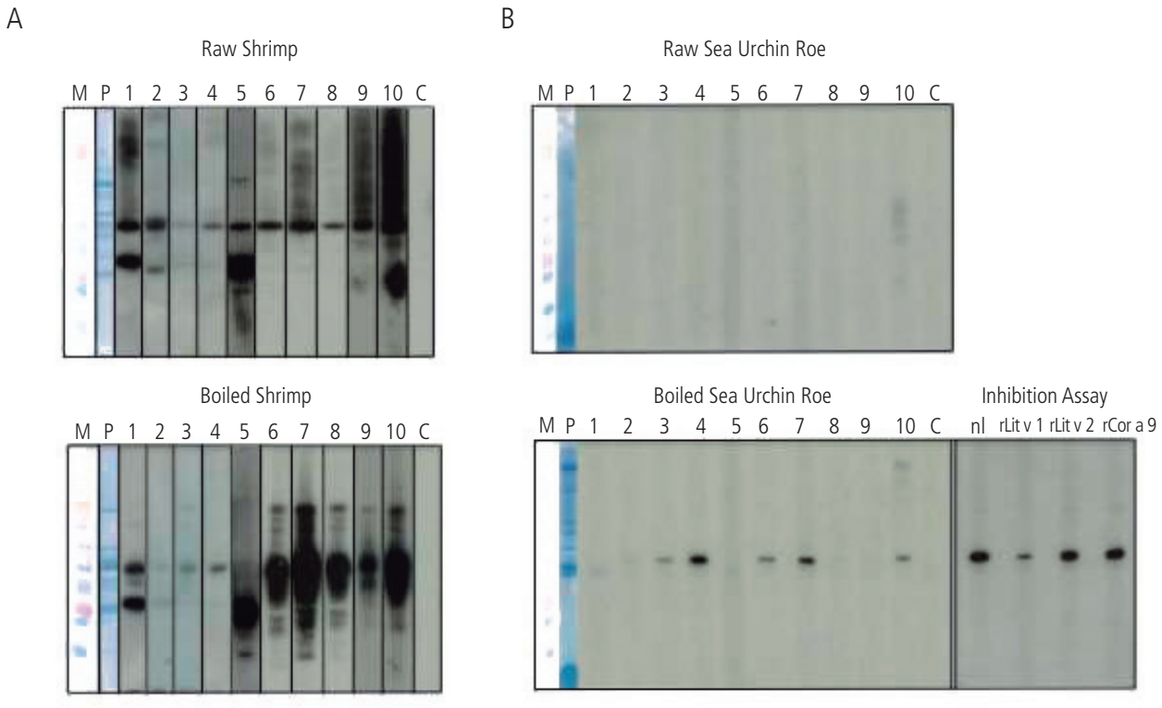

Figure. A, Immunoglobulin E immunoblotting with boiled and raw shrimp extracts. B, Immunoblotting with boiled and raw sea urchin roe extracts, and inhibition assay on boiled sea urchin roe extract. nl indicates noninhibited sera; M, molecular weight; P, protein staining.

concentration was determined with the Coomasie Plus Protein Assay (Pierce). The shrimp and roe proteins separated by SDS-PAGE were electrophoretically transferred onto Immobilon-P membranes (Millipore) [8]. After blocking, the membranes were incubated with sera from individual patients and a nonatopic control. Iodine 125–labeled goat anti-human IgE (DiaMed) was used as the secondary antibody. The membranes were exposed to Kodak Imaging Film (Carestream Health Inc) for 4 to 8 days.

All 10 individuals recognized multiple proteins in the shrimp extracts (Figure 1A), but only 6 showed IgE-binding to a 38-kDa protein in the boiled urchin roe extract. Just 1 individual showed faint recognition of a high molecular weight protein. Almost no protein was recognized in the raw urchin roe (Figure 1B). To identify the immunoreactive urchin protein, an inhibition assay was performed with pooled sera from 3 individuals who recognized the band. Tropomyosin and arginine-kinase, as shrimp allergens of a similar molecular weight that might be implicated in cross-reactivity, were used as inhibitors. The pool (1:20) was preincubated at room temperature for 2 hours with recombinant tropomyosin (Lit v 1), arginine-kinase (Lit v 2), and Cor a 9 as a control (100 ng/μL). Then IgE immunolabeling using a boiled sea urchin roe membrane was performed. Tropomyosin partially inhibited IgE binding (Figure 1B). Several isoforms of tropomyosin have been described in urchin eggs [9]. A tropomyosin-like protein of sea urchin (*Strongylocentrotus purpuratus*) (XP_001192266) showed only 22% sequence identity and 35% similarity with Lit v 1 (ACB38288.1). Such values are lower than those observed between mollusk and shrimp tropomyosins [1]. Since clinical cross-reactivity between shrimp and mollusks is estimated around 15%, for sea urchin it might be less.

Our study shows that some shrimp-allergic patients recognize 1 protein in boiled sea urchin roe and that this protein cross-reacts with shrimp tropomyosin. These patients, therefore, may be at risk of allergic reactions when consuming roe. Since most individuals did not recognize any proteins in raw roe, its ingestion may be safe. An oral challenge with boiled and raw forms of sea urchin roe would help to determine the clinical implications of these findings in shrimp-allergic subjects that wish to consume this food, especially in its boiled form.

References

1. Ayuso R. Update on the Diagnosis and Treatment of Shellfish Allergy. Curr Allergy Asthma Rep. 2011; Apr 15. Online first.
2. How to Read a Label for A Shellfish Free Diet. The Food Allergy & Anaphylaxis Network (FAAN), 2010. Available from: https://www.foodallergy.org/files/media/downloads/HTRLsheet2010.pdf.
3. Asada M, Komura J, Hosokawa H, Akaeda T, Asada Y. A case of delayed hypersensitivity reaction following a sea urchin sting. Dermatologica. 1990; 180(2): 99-101.
4. Rodriguez V, Bartolomé B, Armisén M, Vidal C. Food allergy to Paracentrotus lividus (sea urchin roe). Ann Allergy Asthma Immunol. 2007; 98(4): 393-6.
5. Hickey RW. Sea urchin roe (uni) anaphylaxis. Ann Allergy Asthma Immunol. 2007; 98 (5): 493-4.
6. Damiani E, Nettis E, Priore MG, Delle Donne P, Ferrannini A. Raw Paracentrotus lividus and allergy. Ann Allergy Asthma Immunol. 2008; 101(1): 107-8.
7. Yamasaki A, Higaki H, Nakashima K, Yamamoto O, Hein KZ, Takahashi H, Chinuki Y, Morita E. Identification of a major yolk protein as an allergen in sea urchin roe. Acta Derm Venereol. 2010; 90(3): 235-8.
8. Ayuso R, Grishina G, Bardina L, Carrillo T, Blanco C, Ibañez MD, Sampson HA, Beyer K. Myosin light chain is a novel shrimp allergen, Lit v 3. J Allergy Clin Immunol. 2008; 122 (4): 795–802.
9. Tobita T, Hiraide F, Miyazaki J, Ishimoda-Takagi T. Muscle-type tropomyosin of sea urchin egg increases the actin-binding of nonmuscle-type tropomyosin. J Biochem. 1996; 120(5): 922-8.

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Jessner Lymphocytic Infiltrate as a Side Effect of Bee Venom Immunotherapy

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Key words: Bee sting. Venom immunotherapy side effect. Jessner-Kanof syndrome.

Palabras clave: Picadura de abeja. Efecto secundario de la inmunoterapia frente a venenos. Síndrome de Jessner-Kanof.

Anaphylactic reactions caused by stings are a common medical problem. Onset is acute, with both local and systemic symptoms. In the United States, an estimated 20 to 50 people die every year as a result of severe anaphylaxis after bee, wasp, and ant stings [1].

Allergen immunotherapy has been used in the management of allergy for nearly 100 years. Various venom immunotherapy (VIT) schedules have been designed to treat anaphylaxis [2]. Patients who undergo rapid dose increases and patients treated with bee venom are at increased risk of side effects [3].

We report on a possible side effect of VIT, namely, Jessner lymphocytic infiltrate (also known as Jessner-Kanof syndrome and Jessner pseudolymphoma). This T-cell pseudolymphoma of the skin is an uncommon condition that presents as recurrent asymptomatic papules and plaques with benign coat sleeve–like accumulations of perivascular lymphoid cells on the face, neck, and upper back [4].

A 61-year-old man was followed by our Immunology Service because of an immediate-type reaction to bee sting that was managed with VIT. His medical history was unremarkable, and he reported that he was taking no medications and was a heavy smoker and social drinker.

The patient experienced an anaphylactic reaction 25 minutes after a bee sting, with gradual onset of symptoms. He was admitted to our emergency department in September 2008 with severe dyspnea, wheezing, urticaria, and hypotension (60/35 mmHg). His symptoms began to improve within 2 hours of receiving adrenaline, antihistamines (H_1 and H_2), prednisolone, intravenous infusion fluid, and nasal oxygen supplementation. During the previous year, he had experienced 3 minor local allergic reactions after being stung, although he was not classed as being allergic to venom.

Diagnosis of venom-specific allergy was confirmed by skin tests and elevated venom-specific serum IgE level.

An 8-week VIT regimen was started 45 days after the anaphylactic episode. No premedication was administered, and the initial dose was 0.01 μg, which was increased at weekly intervals. All injections were applied subcutaneously to the outside of the upper arm. The VIT regimen continued with no interruptions of regular weekly injections, although the patient reported symptoms after every injection and severe itching 4-6 hours after the third injection. Physical examination revealed erythematous papules and plaques on his chest and back that lasted for 3 days and subsequently disappeared. He was prescribed antihistamines and topical corticosteroids; however, remission was partial, and the lesions flared.

The type of lesions and their location supported the diagnosis of Jessner lymphocytic infiltrate. The results of a complete blood count, blood biochemistry, and liver function tests were normal, as was the erythrocyte sedimentation rate. The results of autoantibody tests (antinuclear antibody, C3, C4, and rheumatoid factor) were also negative. Histopathology of 1 of the lesions on his back revealed a normal epidermis with no hyperkeratosis, atrophy, or interface changes. The dermis contained moderately dense, perivascular, diffuse infiltrates composed of small, mature lymphocytes that involved the superficial and deep vascular plexuses. The infiltrate extended around the pilosebaceous follicles (Figure, A). Alcian blue staining revealed dermal mucin between the collagen bundles (Figure, B). Periodic acid-Schiff staining revealed no basal vacuolar changes (Figure, C). The cytoplasm and membrane of lymphoid cells stained positive for CD3 using mouse monoclonal antibody (Novocastra, NCL-L-CD3-565 [dilution 1:70]) (Figure, D).

As symptoms worsened after each injection, the VIT protocol was stopped. It was restarted 4 weeks later with premedication consisting of cetirizine dihydrochloride. After the first injection, the patient experienced local reactions, including itching and inflammation (wheal of <5 cm in diameter) around the injection site. No reactions were observed during subsequent sessions, and symptoms had resolved completely at subsequent follow-up visits.

The side effects of VIT range from localized irritation to potentially fatal anaphylaxis. Consequently, clinicians must attempt to prevent such reactions or take all the necessary steps to manage those that do occur [5].

Localized reactions occur in approximately 1 case in every 200 to 500 injections and include transient wheals (urticaria), swelling at the injection site, sneezing, rhinorrhea, and ocular pruritus [6].

Systemic allergic reactions to VIT occur in about 6% of patients, although no cases of Jessner lymphocytic infiltrate or

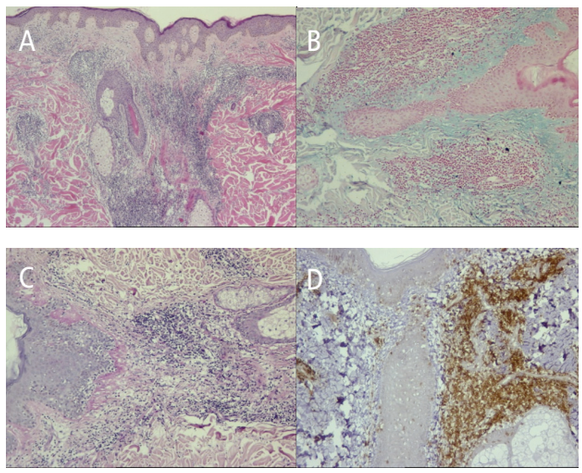

Figure. A, Small lymphocytic infiltrate around the pilosebaceous follicles and perivascular region (hematoxylin-eosin, original magnification × 50). B, Dermal mucin (Alcian blue, original magnification × 100). C, Periodic-acid Schiff stain for normal basal lamina (periodic-acid-Schiff, original magnification × 100). D, CD3 positivity in lymphoid infiltration (mouse monoclonal antibody, × 100).

death due to this procedure have been reported. Systemic reactions may progress to anaphylaxis [3,7].

Jessner lymphocytic infiltrate of the skin can resolve spontaneously without scarring, although it is generally persistent, with an increasing number of lesions. It is also thought to be a dermal variant of systemic lupus erythematosus [4-8]; however, the exact etiology is unknown. In the case we report, systemic lupus erythematosus was ruled out by clinical examination and a negative antinuclear antibody test result. Infection has been suggested as an etiological factor, as has photosensitivity, since exposure to sunlight can irritate or aggravate the disease [4-8]. Familial cases are rare [9].

Our patient was diagnosed with Jessner lymphocytic infiltrate 3 weeks after initiation of VIT. His lesions flared after each injection, thus indicating an association between therapy and the disease. The fact that the lesions resolved when treatment was stopped seems to support this association.

To our knowledge, this is the first report of Jessner lymphocytic infiltrate as a side effect of venom immunotherapy. We urge clinicians to be aware of this side effect in similar cases.

References

1. Barnard JH. Studies of 400 Hymenoptera sting deaths in the United States. J Allergy Clin Immunol. 1973;52(2):259-64.
2. Bilò MB. Anaphylaxis caused by Hymenoptera stings: from epidemiology to treatment. Allergy. 2011;66:35-7.
3. Mosbech H, Müller U. Side-effects of insect venom immunotherapy: results from an EAACI multicenter study. European Academy of Allergology and Clinical Immunology. Allergy. 2000;55:1005-10.
4. Toonstra J, Wildschut A, Boer J, Smeenk G, Willemze R, van der Putte SC, Boonstra H, van Vloten WA. Jessner's lymphocytic infiltration of the skin. A clinical study of 100 patients. Arch Dermatol. 1989;125:1525-30.
5. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Muller U; EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy. 2005;60:1459-70.
6. Grammer LC, Greenberger PA. Patterson's Allergic Diseases, 6th Edition. Philadelphia; Lippincott Williams & Wilkins; 2002.
7. Birnbaum J, Ramadour M, Magnan A, Vervloet D. Hymenoptera ultra-rush venom immunotherapy (210 min): a safety study and risk factors. Clin Exp Allergy 2003;33:58-64.
8. Lipsker D, Mitschler A, Grosshans E, Cribier B. Could Jessner's lymphocytic infiltrate of the skin be a dermal variant of lupus erythematosus? An analysis of 210 cases. Dermatology. 2006;213:15-22.
9. Dippel E, Poenitz N, Klemke CD, Orfanos CE, Goerdt S. Familial lymphocytic infiltration of the skin: histochemical and molecular analysis in three brothers. Dermatology. 2002;204:12-6.

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Allergy to Mint (*Mentha spicata*)

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Key words: *Mentha spicata*. IgE-mediated. Prick by prick. Food allergy.

Palabras clave: *Mentha spicata*. Mediado por IgE. Prick-prick. Alergia alimentaria.

Mentha spicata is a member of the *Lamiaceae* family (formerly, Labiateae), which also includes other aromatic plants of genera such as *Thymus vulgaris* (thyme), *Rosmarinus officinalis* (rosemary), *Ocimum basilicum* (basil), and *Origanum vulgare* (oregano). The genus *Mentha* includes 20 to 30 true species, as well as numerous hybrids, the best-known of which are *M spicata* (hybrid between *Mentha suaveolens* and *Mentha longifolia*) and *Mentha piperita* (which results from a cross between *M spicata* and *Mentha aquatica*) [1]. *M spicata* is a common evergreen plant that grows all over Europe and the United States. The essential oil extracted from its leaves is widely used in the food, pharmaceutical, and cosmetics industries [2].

Several cases of mint hypersensitivity have been reported in the literature, including type IV hypersensitivity reactions caused by contact with mint-flavored toothpaste [3], application of compresses soaked in fresh mint leaf infusion [2], and ingestion of tea brewed from leaves of *Mentha pulegium* [4], as well as occupational contact urticaria, caused by *M pulegium* [1]. The culprit allergens in these cases were carvone, limonene, and menthol [2].

Fresh leaves of *M spicata* are frequently used in salads and in cooked foods; however, immunoglobulin (Ig) E–mediated reactions after ingestion of mint seem to be rather unusual. Paiva et al [5] recently described a case of IgE-mediated anaphylaxis caused by *M piperita* associated with toothpaste.

We present the case of a 41-year-old female teacher with a history of rhinoconjunctivitis associated with exposure to house dust mite and cat. The patient was referred to our department for recurrent episodes of uvular angioedema that appeared a few hours after ingestion of fresh mint leaves (used as a condiment) or inhalation of mint essence during a massage. The reactions resolved with antihistamine treatment. Notably, the patient did not report a history of reactions related to ingestion of other foods, including aromatic herbs from the mint family, or cutaneous reactions in the areas where the mint essence was applied.

Skin prick tests were performed using commercial extracts of common food allergens and aeroallergens (Stallergènes), with positive results for *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and dog and cat epithelium. Prick-by-prick testing was performed with fresh mint leaves, since neither a commercial mint extract for prick testing nor radioallergosorbent tests (RAST) were available. The results of prick-by-prick testing and RAST with substrates of other members of the *Lamiaceae* family (oregano, rosemary, basil, and thyme) were all negative. Moreover, in order to achieve a more complete diagnosis, patch tests were performed with the standard series of the Italian Society

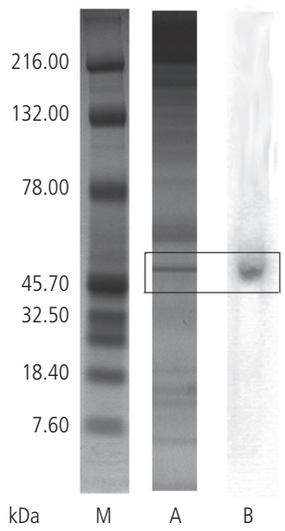

Figure. Immunoblotting analysis of *Mentha spicata* protein extract. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of 27 μg of extract performed under reducing conditions (80 V, 2 hours) revealed approximately 18 discrete bands (lane A). After blotting to a nitrocellulose membrane, which was incubated overnight at 4°C with the patient's serum, addition of the secondary antibody revealed a single immunoreactive band, with an apparent molecular weight of 50 kDa (lane B). Lane M, molecular markers (Kaleidoscope prestained standards, broad range, BioRad).

of Allergology, Occupational and Environmental Dermatology (SIDAPA), fresh mint leaves, and mint extract. The results of all tests were negative. The patient refused to undergo a bronchial provocation test with mint essence.

The prick-by-prick test and patch test with fresh mint leaves and mint extract, respectively, were also performed in 10 healthy controls, with negative results.

A protein extract was then obtained from mint leaves (5.96 mg/mL). After staining with Coomassie Colloidal Blue 0.1%, the gel electrophoresis profile (Bis Tris Nupage, Invitrogen) revealed bands of molecular weight ranging from approximately 6 to 62 kDa (Figure).

For immunoblotting, the proteins were transferred to a nitrocellulose membrane (Schleicher & Schuell BioScience) (pore size 0.45 μm; 25 V for 1.5 hours)—saturated as described elsewhere [6]—and incubated overnight with the patient's serum. Goat antihuman IgE antibody conjugated with peroxidase (1:1000 in saturation buffer) was then added. After 2 hours, specific IgE binding was revealed by chemiluminescence, showing a single immunoreactive band of approximately 50 kDa (Figure).

In conclusion, the positive prick-by-prick results with fresh mint leaves and specific IgE binding to a protein with a molecular weight of approximately 50 kDa detected by immunoblotting led us to assume that an IgE-mediated mechanism was responsible for the reactions experienced by our patient. Therefore, as a preventive measure, the patient was advised to avoid both ingestion of fresh mint leaves and exposure to preparations in which mint is used as a flavoring ingredient. Finally, to our knowledge, this is the first time that a 50-kDa protein has been recognized as an allergen in fresh mint. Further allergen characterization studies are necessary in order to determine whether this protein could be proposed as a major mint allergen.

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References

1. Pérez-Calderon R, Gonzalo-Garijo A, Bartolomé-Zavala B, Lamilla-Yerga A, Moreno-Gastón I. Occupational contact urticaria due to pennyroyal (Mentha pulegium). Contact Dermatitis. 2007;57:285-6.
2. Bonamonte D, Mundo L, Daddabbo M, Foti C. Allergic contact dermatitis from Mentha spicata (spearmint). Contact Dermatitis. 2001;45:298.
3. Holmes G, Freeman S. Cheilitis caused by contact urticaria to mint flavoured toothpaste. Australas J Dermatol. 2001;42:43-5.
4. Roé E, Serra-Baldrich E, Dalmau J, Peramiquel L, Pérez M, Granel C, Alomar A. Mentha pulegium contact dermatitis. Contact Dermatitis. 2005;53:355.
5. Paiva M, Piedade S, Gaspar A. Toothpaste-induced anaphylaxis caused by mint (Mentha) allergy. Allergy. 2010;65:1201-2.
6. Damiani E, Aloia AM, Priore MG, Nardulli S, Ferrannini A. Pomegranate (Punica granatum) allergy: clinical and immunological findings. Ann Allergy Asthma Imunol. 2009;103:178-80.

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Variations in TGF-ß, IL-10, and IL-17 After Specific Immunotherapy and Correlations With Symptoms in Patients With Allergic Rhinitis

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Interleukin (IL) 10, IL-17, and transforming growth factor (TGF) ß play an important role in the pathogenesis of allergic rhinitis (AR); however, their role in specific immunotherapy (SIT) for AR and correlation with symptoms remain unknown. Our study analyzed nasal symptoms and compared variations in IL-10, TGF-ß, and IL-17 levels before and after SIT in patients with AR in order to assess their role in SIT.

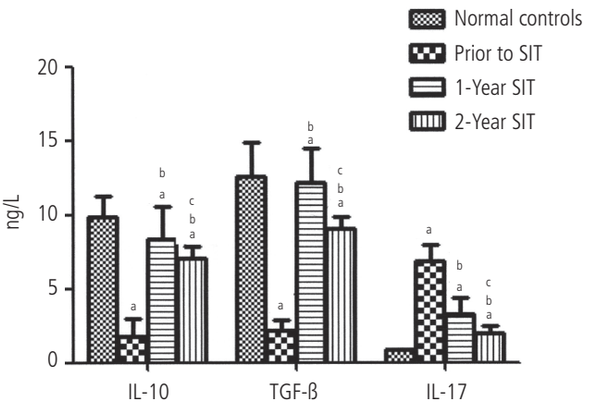
The study population comprised 48 patients (26 males and 22 females) aged 7 to 42 years (mean, 23.5 years) with perennial AR from the Otolaryngology Department of Guangdong General Hospital from March to December, 2008. The diagnostic criteria applied were from 'Principles of diagnosis, therapy and recommendation for allergic rhinitis,' drafted at the 2004 Lanzhou Convention [1]. Skin prick test results were positive mainly for house dust mite. Patients with an intense positive response (++++) to *Dermatophagoides pteronyssinus* (Der p) had positive responses to allergens graded lower than ++, including pollens in spring and, autumn, multiple fungi, and cockroach. Determination of specific immunoglobulin (Uni-CAP, Pharmacia) revealed grade ≥3 Der p3–specific IgE in serum. The control group comprised 35 healthy individuals (22 males and 13 females) aged 13 to 41 years (mean, 26.5 years) who had no relevant history of allergy.

A 2-year course of therapy for *Dermatophagoides pteronyssinus* allergen (Alutard, ALK-Abelló) was administered subcutaneously at an initial dose of 20 SQ-U that was gradually increased to 100 000 SQ-U per week in the primary stage. The 100 000 SQ-U dosing interval was extended to 6 weeks in the maintenance stage.

Symptoms were scored during enrollment and 1 and 2 years after SIT (mean daily score over 1 week). Patients reported nasal congestion, nasal pruritus, sneezing, and rhinorrhea (scores of 0, 1, 2, and 3 defined as no symptoms, mild symptoms, moderate symptoms, and severe symptoms, respectively). The total nasal symptom score was defined as the sum of each item. Serum IL-10, TGF-ß, and IL-17 were detected using enzyme-linked immunosorbent assay (ELISA) at baseline (before therapy started) and 1 and 2 years after SIT.

The mean (SD) symptom score ($P<.05$) in patients with AR was (9.73 [1.22]), (4.42 [1.36]), and (3.91 [1.29]) at baseline and at 1 and 2 years of SIT. Variations in IL-10, TGF-ß, and IL-17 at baseline and at 1 and 2 years are shown in the Figure.

According to Pearson's model, a negative correlation was found between levels of serum IL-10 and TGF-ß ($r=–0.41$, $P<.05$; $r=–0.33$, $P<0.05$) and symptom scores before SIT in the patients.



[a]Compared with normal controls, $P<.05$
[b]Compared with AR patients prior to SIT, $P<.05$
[c]Compared with AR patients 1 year after SIT, $P<.05$

Figure. Variations in IL-10, TGF-ß, and IL-17. SIT indicates specific immunotherapy; IL, interleukin; TGF, transforming growth factor.

A negative correlation was also found between serum IL-10 and TGF-ß ($r=–0.34$, $P<.05$; $r=–0.31$, $P<.05$) and symptom scores at 2 years after SIT. However, no correlation was found between these levels and symptom scores at 1 year after SIT ($P>.05$). The level of IL-17 was positively correlated with symptom scores ($r=0.57$, $P<.05$) and maintained the positive correlation at 2 years ($r=0.52$, $P<.05$). However, no correlation was revealed at 1 year ($P>0.05$). Our results indicate that IL-10, TGF-ß, and IL-17 could serve as indicators to assess the efficacy of SIT at 2 years.

The discovery of regulatory T cells (Treg) and type 17 helper T cells (T_H17) complemented the T_H1/T_H2 balance hypothesis [2]. As the major immunosuppressive cytokines secreted by Tregs, IL-10 and TGF-ß exhibit extensive action by inhibiting the release of proinflammatory cytokines, including suppression of antigen-presenting cells, and indirectly inhibiting cellular response and secretion of specific IgE. Additionally, both cytokines could stimulate synthesis of IgG4 and downregulate airway inflammation, thus suppressing the development of AR. In contrast, downregulation of IL-10 and TGF-ß in vivo could lead to allergic disorders [3]. The decrease in serum IL-10 and TGF-ß levels, together with the negative correlation with nasal symptoms, suggests that low expression of both cytokines might cause nasal inflammation in individuals with AR.

IL-17 is the major cytokine released by T_H17 lymphocytes, which have proinflammatory and chemotactic bioactivity [4,5]. Ciprandi et al [6] reported a close correlation between serum IL-17 and clinical symptoms in AR patients who were allergic to birch and proposed applying serum IL-17 level as the indicator of severity of allergy. We observed a positive correlation between the nasal symptom score and a higher serum IL-17 level in AR patients who were allergic to house dust mite, suggesting that cytokine level might serve as an indicator of severity of allergy.

As for treatment, SIT remains the only available approach that alters the natural progress of allergic disorders through immunomodulation [7]. Since the local cytokine profile is more likely to change, serum cytokine level may better reflect the condition of the immune system. Increased serum IL-10 level is considered indicative of the success of SIT, while the role of TGF-ß remains open to debate [8]. As our results indicate, a significant improvement in serum IL-10 and TGF-ß levels was observed over

a 2-year treatment period compared with levels at baseline. The negative correlation with symptoms indicates that both cytokines are involved in immunomodulation, thereby diminishing airway hyperresponsiveness and suppressing allergic inflammation. However, the expression of both IL-10 and TGF-ß after 2 years of treatment was lower than after 1 year of treatment, probably because of the gradual return to a balanced immune system and the relatively low baseline level. The reduction in IL-17 expression at 2 years of SIT compared with 1 year indicated that immunotherapy might downregulate expression of IL-17. Besides, the finding of lower IL-10, TGF-ß, and IL-17 levels than in controls after 2 years of treatment seemed to be correlated with the re-establishment of immunity over time. Longer duration of immunotherapy could be associated with a more significant improvement in symptoms and superior efficacy. In conclusion, IL-10, TGF-ß, and IL-17 play a vital role in the pathogenesis of AR and the response to SIT. Factors leading to variations and underlying mechanisms should be further investigated.

References

1. The otolaryngoharyngology branch of the Chinese Medical Association, the editing board of The Chinese Journal of Otolaryngopharyngology: Principles of diagnosis, therapy and recommendation for allergic rhinitis (2004 Lanzhou Convention). The Chinese Journal of Otolaryngopharyngology. 2005;40:166-7.
2. Akdis CA, Akdis M. Mechanisms and treatment of allergic disease in the big picture of regulatory T cells. J Allergy Clin Immunol. 2009;123:735-46.
3. Prigione I, Morandi F, Tosca MA, Silvestri M, Pistoia V, Ciprandi G, Rossi GA. Interferon-gamma and IL-10 may protect from allergic polysensitization in children: preliminary evidence. Allergy. 2010;65:740-2.
4. Kolls JK, Linden A. Interleukin-17 family members and inflammation. Immunity. 2004;21(4):467-76.
5. Hung LY, Velichko S, Huang F, Thai P, Wu R. Regulation of airway innate and adaptive immune responses: the IL-17 paradigm. Crit Rev Immunol. 2008;28(4):269-79
6. Ciprandi G, De Amici M, Murdaca G, Fenoglio D, Ricciardolo F, Marseglia G, Tosca M. Serum interleukin-17 levels are related to clinical severity in allergic rhinitis. Allergy. 2009;64:1375-8.
7. Alvarez-Cuesta E, Bousquet J, Canonica GW, Durham SR, Malling HJ, Valovirta E; EAACI, Immunotherapy Task Force. Standards for practical allergen-specific immunotherapy. Allergy. 2006;61(Suppl 82):1-20.
8. Ajduk J, Marinic I, Aberle N, Rabatic S, Gagro A. Effect of house dust mite immunotherapy on transforming growth factor beta1-producing T cells in asthmatic children. Ann Allergy Asthma Immunol. 2008;100:314-22.

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