In Vitro Production of Ag-Specific IFN-γ in Patients With Delayed Hypersensitivity to Amoxicillin

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In recent years many studies have investigated the production of cytokines in the supernatants of cell cultures from patients with delayed hypersensitivity reactions to drugs as an in vitro tool for the diagnosis of reactions of this type. The detection of cells producing interferon (IFN) γ using ELISPOT [1,2] or the quantification of IFN-γ produced both at the intracellular level [3] and in the supernatants of cell cultures [4-6] are the diagnostic methods that have received most attention. However, results have been highly variable, with sensitivity rates ranging from 26% to 91% and specificity rates ranging from 60% to 100%.

We analyzed 15 patients with delayed allergic reactions to amoxicillin diagnosed by oral challenge and/or patch tests and/or intradermal tests with delayed reading (Table). An oral challenge was not performed in 1 patient (#13) for

Table. Medical Profile and Diagnostic Tests of Patients With Delayed Hypersensitivity Reactions to Amoxicillin. In Vitro Results for Patients and Healthy Controls, With Interferon (IFN) γ Production Expressed as the Stimulation Index

Patients	Age, y	Sex	Reaction Type	Time Since Reaction, mo	Diagnostic Test	Stimulation Index[a]		
						WB	PBMCs	PBMC+PHA (ELISA/CBA)
1	19	F	GCE	11	PT	0.93	1.81	n.d.
2	46	M	GCE, Ed	96	OC*	1.05	0.90	n.d.
3	38	F	GCE	2	OC+	n.d.	1.39	n.d.
4	65	M	Exf Der	40	LIST	0.82	1.00	n.d.
5	35	F	GCE, Ed	170	OC+	**4.74**	1.73	n.d.
6	65	F	Exf Der	42	OC+	**3.06**	2.72	n.d.
7	48	F	GCE	114	OC+	**8.22**	0.88	n.d.
8	12	F	GCE	1	OC+	**2.22**	1.21	n.d.
9	4	F	GCE	2	OC+	1.10	2.55	n.d.
10	37	F	MFE	6	OC+	0.57	1.37	n.d.
11	66	F	GCE	53	PT, LIST	1.15	0.97	0.67/**93.00**
12	50	F	GCE	360	OC+	n.d.	1.17	**2.38/11.77**
13	47	F	LV	7	SB, 2 times	n.d.	n.d.	**2.05/2.49**
14	64	F	Exf Der	50	OC+	n.d.	n.d.	**7.84/5.20**
15	72	F	EEM	60	EC	n.d.	n.d.	**3.09/2.23**
Controls								
1	33	M	–	–	OC-	1.29	1.00	0.82/1.21
2	26	F	–	–	OC-	1.02	**2.05**	**28.19**/1.62
3	55	F	–	–	OC-	1.00	**2.38**	0.59/0.05
4	30	F	–	–	OC-	2.00	**3.96**	n.d.

Abbreviations: Ed, edema; EEM, exudative erythema multiforme; Exf Der, exfoliative dermatitis; GCE, generalized cutaneous exanthema; MFE, multilocular fixed exanthema; LIST, late intracutaneous skin test; LV, leukocytoclastic vasculitis; n.d., not done; OC+, positive oral challenge test; OC-, negative oral challenge test; PBMC, peripheral blood mononuclear cell; PBMC+PHA, PBMC stimulated with phytohemagglutinin; PT, patch test; SB, skin biopsy; WB, whole blood.
[a]Calculated as IFN-γ quantified in the supernatants of the cell cultures stimulated in the presence of amoxicillin divided by that quantified in the supernatants of cell cultures without stimulation.

ethical reasons, as leukocytoclastic vasculitis was detected on 2 occasions on the third day following administration of amoxicillin. All the patients provided informed consent before the collection of blood for the analysis of IFN-γ production in response to amoxicillin. With the aim of finding a rapid and simple in vitro method, we analyzed the production of IFN-γ following Ag-specific stimulation in the supernatants of samples of whole blood (WB)) (24 hours, 37º C, 5% CO_2) and of cultures of mononuclear cells isolated from peripheral blood (PBMCs) (0.5×10^6 cells/mL in RPMI 1640 + 10% FCS, 72 hours, 37º C, 5% CO_2) from 10 patients with delayed allergic reactions to amoxicillin (Table) and 4 healthy controls. We then analyzed, based on the method previously described by Halevy and Grossman [6], Ag-specific production of IFN-γ in the supernatants of cultures of PBMCs stimulated under suboptimal conditions with phytohemagglutinin A (PHA) (0.5×10^6 cells/mL in RPMI 1640 + 10% FCS + 5 μg/mL PHA, 72 hours, 37º C, 5% CO_2) from 5 patients with delayed allergic reactions to amoxicillin and 3 healthy controls. Cells were cultured in duplicate and stimulated with 3 different concentrations of amoxicillin (0.5, 0.2, and 0.1 mg/mL); 10 μg/mL of PHA was used as a positive control and basal conditions (without amoxicillin) served as a negative control. The IFN-γ in the supernatants of the cell cultures was quantified using 2 techniques in parallel: enzyme-linked immunosorbent assay (ELISA) (Human IFN-γ Opt EIA, BD Bioscience), and flow cytometry (Cytometric Bead Array [CBA] Human Th1/Th2/Th17 Cytokine Kit; BD Bioscience). Production of IFN-γ was calculated using a stimulation index (SI) (IFN-γ quantified in the supernatants of the cell cultures stimulated in the presence of amoxicillin divided by that quantified in the supernatants of cell cultures without stimulation). Sensitivity, specificity, area under the curve (AUC), and the optimal cutoff for sensitivity and specificity were estimated using receiver operator curves. Sensitivity and specificity values were calculated for each of the 3 types of cultures (WB, PBMCs, and PBMCs+PHA).

The Table summarizes the results obtained. The optimal cutoff was that where the SI was greater than 2 for the 3 types of culture. When IFN-γ was quantified using ELISA, the AUC for the SI was 0.55 for the WB cultures, 0.26 for the PBMC cultures, and 0.60 for the PBMC+PHA cultures. However, when quantification was performed using CBA, the AUC was 1.

The main objective of this study was to test the quantification of IFN-γ in the supernatants of WB stimulated with a drug for 24 hours as a rapid and simple method for the in vitro diagnosis of delayed hypersensitivity reactions to drugs. This technique yielded a specificity of 100% but sensitivity was low (40%). Similar sensitivity values have been reported for PBMCs cultured for 72 hours [3,7] but higher values, in the range of 80% to 100%, have also been reported [4,5]. Our results for the PBMC cultures were worse in terms of both sensitivity (17%) and specificity (25%). The best diagnostic values were obtained with the quantification of IFN-γ secreted into the medium of PBMC cultures stimulated under suboptimal conditions with 5 μ/mL of PHA; this is similar to the method used by Halevy and Grossman [6], although they used a larger quantity of PHA (200 μg/mL). When we quantified IFN-γ using ELISA, we obtained a specificity of 67% and a sensitivity of 80%; CBA, however, yielded a specificity and sensitivity of 100%. The different results can be explained by the small amount of IFN-γ quantified in the supernatants of the cell cultures from patient 11 and control 2 at baseline using CBA and ELISA, respectively.

We conclude that the culture method providing the best results for the diagnosis of delayed hypersensitivity reactions to amoxicillin is quantification of IFN-γ production following Ag-specific stimulation for 72 hours using CBA in cultures of PBMCs stimulated in suboptimal conditions with 5 μg/mL of PHA. Further studies are needed to analyze the conditions assayed in our study with a larger group of patients.

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Baseline Tryptase Levels Are Related to Age, Total IgE, and Anti-rPru p 3 IgE Levels in Peach-Allergic Patients

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Tryptase and histamine are preformed mediators released by mast cells upon activation. Since tryptase persists for hours after an anaphylactic event, an increase in tryptase levels is currently considered the best available marker of anaphylaxis. Mast cells also constitutively secrete ß-tryptase, the serum levels of which reflect mast-cell burden. Accordingly, high levels of ß-tryptase suggest the presence of systemic mastocytosis. Moreover, moderately increased levels of tryptase associated with urticaria, flushing, headache, and/or gastrointestinal reactions in the absence of World Allergy Organization mastocytosis criteria were recently classified as mast cell activation syndrome (MCAS). In allergy, apart from acute reactions, high tryptase levels have been reported only in hymenoptera venom allergy, in association with an increased risk of severe anaphylaxis from both stings and venom immunotherapy [1]. Hymenoptera venom allergy is also associated with mastocytosis [2]. Whether elevated baseline serum tryptase levels might also be a risk factor for severe reactions in food allergy is unknown. Lipid transfer protein (LTP), a plant panallergen [3], is the major cause of primary food allergy and food-induced anaphylaxis in Mediterranean countries [4,5]. The clinical expression of LTP hypersensitivity shows much variability, ranging from the total absence of symptoms to oral allergy syndrome (OAS), isolated gastrointestinal symptoms, urticaria/angioedema, and even severe anaphylaxis. The cause of such variability is unclear and only partially explained by LTP-specific immunoglobulin (Ig) E levels [6]. Nonetheless, an association between high levels of anti-Pru p 3 (LTP) IgE and peach allergy severity may exist and cosensitization to Pru p 1 seems to attenuate clinical reactivity [7]. In this study we measured baseline serum tryptase levels in peach-allergic individuals and looked for a possible association with clinical variables (symptom severity, sex, age) and immunological variables (total IgE and Pru p 3 IgE levels).

We studied 148 peach-allergic adults (41 males/107 females [P<.001]; median age, 37 years; range, 13-62 years) [7]. Seventy-six had OAS as the only clinical expression of peach allergy, whereas 72 had both OAS and systemic symptoms of increasing severity (classified as OAS grades II, III, and IV) [7]. The patients had different sensitization profiles to rPru p 1 (the Bet v 1 peach homologue), Pru p 3 (LTP), and Pru p 4 (profilin). Serum tryptase was related to age, anti-rPru p 3, anti-rPru p 1, total IgE levels, symptom severity, and number of sensitizing foods other than peach (apricot, cherry, apple, pear, lemon, orange, fennel, grape, strawberry, peanut, nut, hazelnut, chestnut, wheat, rice, maize, tomato, garlic, onion, kiwi, celery, lettuce, green bean, pea, and soy). Forty healthy individuals (median age, 39 years) served as negative controls. Specific IgE was measured by ImmunoCAP 1000 (Thermofisher/Phadia). Tryptase was measured on a single occasion in each patient by immunofluorometric assay (ImmunoCAP 100) during an asymptomatic period, at least 4 months after the last anaphylactic event. Although levels exceeding 11.4 ng/mL are usually considered sufficient to diagnose possible mastocytosis, in this study we considered absolute tryptase levels. Correlations between IgE levels were assessed using Spearman rank correlation (ϱ). Associations between the number of sensitizing foods and anti-rPru p IgE levels and between tryptase levels and sex and symptom severity were analyzed by the Mann-Whitney U test. The variation of tryptase in function of age, total IgE, and anti-rPru p IgE levels was analyzed by linear regressions with robust standard error. The study was a secondary endpoint of a previously published study [7] (Clin Trials gov protocol ID NCT00715156).

The median tryptase level in patients was 2.48 ng/mL (range, 0-12.2 ng/mL). Levels exceeded 11.4 ng/mL and 5 ng/mL in only 2 (1%) and 15 (10%) cases, respectively. In the controls, the median tryptase level was 3.29 ng/mL. Mean (SD) tryptase levels were 2.88 (2.19) ng/mL, 2.78 (2.18) ng/mL, 2.75 (2.78) ng/mL, and 3.88 (2.25) ng/mL in patients with OAS grades I, II, III and IV, respectively. No significant differences were observed between OAS grades or between patients and controls. There was also no correlation between tryptase levels and severity of peach allergy (P=.63), although 1 male patient with a level of 11 ng/mL experienced the most severe reactions. Univariate regression analysis using tryptase as the dependent variable showed tryptase to be significantly related with age (P=.025; 95% CI, 0.002-0.056), total IgE levels (P=.015; 95% CI, 0.000-0.002), and rPru p 3 IgE levels (P=.032; 95%CI, 0.002-0.057). The figure shows the scatterplot and robust regression line of tryptase levels versus anti-rPru p3 IgE levels. In the multivariate regression model with tryptase as the dependent variable, tryptase levels were significantly related to age (P=.034; 95% CI, 0.002-0.056) and total IgE levels (P=.020; 95% CI, 0.000-0.002), confirming the interrelationship between these parameters. Furthermore, the number of sensitizing plant foods other than peach was higher in rPru p3-positive patients than in rPru p3-negative patients (P<.0001). No associations were found between tryptase and IgE to rPru p1 or rPru p4.

To date elevated baseline tryptase levels have been associated with severe systemic reactions due to insect stings or venom immunotherapy, but not with food allergy. In

our patients, tryptase levels were normal and only correlated with the severity of peach allergy in 1 case. We found a significant interrelationship between tryptase and age, total IgE, and anti-Pru p 3 IgE levels. The relationship with age has already been described in venom anaphylaxis, and indeed age seems to be among the main risk factors for fatal anaphylaxis in drug allergy. However, in venom allergy the severity of reactions is particularly related to baseline tryptase levels, which does not seem to be the case for systemic reactions due to injection immunotherapy [8], drug allergy, or food allergy [9]. In this study, only rPru p 3 IgE levels were related to symptom severity. The most interesting finding was the relationship between tryptase and anti-Pru p 3 IgE levels. As high Pru p 3 IgE levels are correlated with sensitization to an array of clinically tolerated plant-derived foods due to cross-reactivity [10], it is likely that the repeated ingestion of clinically tolerated plant foods to which patients are sensitized may induce a minimal but appreciable mast-cell stimulation mirrored by a slight increase in tryptase levels.

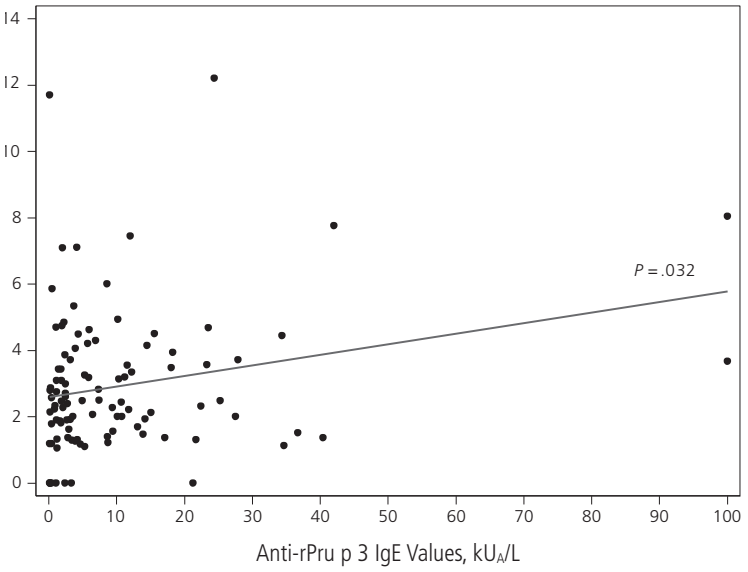

Figure. Scatterplot and robust regression line of tryptase levels versus anti-rPru p 3 IgE levels showing the *P* value (.032) for the regression coefficient. IgE indicates immunoglobulin E.

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Recurrent Angioedema Associated With Secondary Eosinophilia

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Palabras clave: Angioedema. Eosinophilia. *Ascaris lumbricoides*. *Anisakis*.

Episodic angioedema with eosinophilia was first described by Gleich et al [1] in 1984. Since then, several cases have been reported [2,3]. Clinical presentation is characterized by recurrent episodes of angioedema, urticaria, pruritus, and peripheral blood eosinophilia. To the best of our knowledge, angioedema has not been associated with secondary eosinophilia, such as that occurring during parasitic diseases, which result from infestation by helminths, protozoa, or arthropods and are usually characterized by constant or fluctuating eosinophilia. Parasites stimulate production of interleukin (IL) 5, which drives eosinophil expansion, a key factor in the elimination of parasites [4]. Symptoms vary, depending on the type of parasite and the organ involved. In addition, a hypercoagulable state has been described [5].

A 49-year-old man was admitted to our department with angioedema, arthralgia, and itching. Upon admission, he was afebrile with a pulse rate of 83 beats/min, respiratory rate of 16 breaths/min, and blood pressure of 135/85 mmHg. His body mass index was 28.3 kg/m^2. Physical examination revealed no abnormalities, except for localized swelling with wheals on the volar aspect of the forearm; this type of lesion recurred 3-4 times a month on different parts of the body (thighs, trunk, and neck). Symptoms had started 4 months earlier. The patient was not taking chronic treatment at home, except for oral H_1 antihistamines and corticosteroids during angioedema attacks. He had no history of smoking, drinking, or allergy. He often ate raw fish and had travelled to the Middle and Far East. The results of ECG, chest X-ray, abdominal ultrasonography, and echocardiography were all normal. Laboratory tests showed hypereosinophilia (3260/μL [27.5%]), with high serum total immunoglobulin (Ig) E (843 kU/L), and very high serum eosinophil cationic protein levels (at admission, 210 μg/L; normal values, <4 μg/L). Serum protein electrophoresis was normal, and his stools were free of parasites, their eggs, and *Helicobacter pylori* antigen. Values for C-reactive protein, erythrocyte sedimentation rate, tryptase, C3 and C4, C1-inhibitor antigen, and C1-inhibitor function were within the normal range. The search for antinuclear antibodies, anti-extractable nuclear antigens, cytoplasmic and perinuclear antineutrophil cytoplasmic antibodies, anticyclic citrullinated protein antibodies, and anti–double-stranded DNA was negative, except for weak rheumatoid factor positivity. Screening for viral infection (hepatitis B virus, hepatitis C virus, and human immunodeficiency virus) was negative. Serology for cytomegalovirus and Epstein-Barr virus was negative for acute infection, and the Widal-Wright and Weil-Felix reactions were also negative. No anti-*Strongyloides* or anti–*Toxocara canis* antibodies were detected, but levels of IgE specific for *Ascaris lumbricoides* and *Anisakis* were high (14.3 kU_A/L and 9.48 kU_A/L, respectively; reference values, <0.1 kU_A/L for both). The findings of esophagogastroduodenoscopy (performed to evaluate the presence of parasites in the gastric mucosa) were normal. Bone marrow biopsy revealed an increased number of eosinophilic myeloid cells, with no increase in the immature cell count. The results of tests to detect mutations associated with idiopathic hypereosinophilic syndrome (analysis of the *FIP1L1-PDGFRA* and *WT1* genes) were negative. Given the high serum levels of specific IgE to *Ascaris* and *Anisakis*, we started anthelminthic treatment with albendazole at a dose of 100 mg bid for 3 days. A checkup after 14 days disclosed persistently high peripheral blood eosinophil counts; thus, the patient was treated with a more effective antinematodal drug (ivermectin, 18 mg in a single dose). Eosinophil counts decreased progressively to normal values within 15 days (Figure). Treatment with ivermectin was repeated after 1 month. One month later, clinical and blood examinations revealed absence of angioedema and a lasting normal eosinophil count. Notably, the very high initial serum levels of eosinophil cationic protein (>200 μg/L) had returned to normal. Serum specific IgE to *Ascaris* and *Anisakis* had decreased 2- to 3-fold (7 and 3 kU_A/L, respectively). A subsequent checkup 5 months later showed that the patient was still free of angioedema, with a persistently normal eosinophil count.

We observed an association between recurrent angioedema and persistent peripheral blood eosinophilia. The complete resolution of both angioedema and eosinophilia following anthelminthic treatment and the high levels of specific IgE to *A lumbricoides* and *Anisakis* led us to consider a diagnosis of eosinophilia secondary to a parasitic infestation, even though we could not detect the nematodes directly. Although the stool examination for parasites and their eggs was negative and no parasite was detected in the duodenum, a diagnosis of ascaridiasis cannot be ruled out. The sensitivity of the Ritchie concentration method [6] used to detect helminths and their eggs is variable, and false-negative results can occur. The strong positivity of IgE antibodies against *A lumbricoides* and the response to anthelminthic treatment indirectly demonstrates infestation by this parasite. On the other hand, infestation by *Anisakis* cannot be completely ruled out, as direct detection of the parasite is very difficult [7]. In addition, the patient reported that he frequently ate raw fish and that he had specific IgE to *Anisakis* (Phadia AB). However, serum levels of specific

IgE to *Anisakis* were lower than those of IgE to *Ascaris* (Phadia AB). The finding of specific IgE to *Anisakis* might be the result of cross-reactivity with *Ascaris* antigens, as indicated in the literature [8]. The patient recovered after receiving ivermectin, which is very effective against nematodes [9]; albendazole was initially ineffective. Irrespective of the parasite involved, it is interesting to note that anthelminthic therapy completely resolved both eosinophilia and angioedema. This observation lends further support for the role of eosinophils in the pathophysiology of angioedema. Eosinophils can induce angioedema directly by releasing inflammatory mediators that increase vascular permeability, such as leukotriene C_4, platelet-activating factor, and vascular endothelial growth factor [10]; however, they can also act indirectly by releasing major basic protein, which can activate mast cells, leading to release of inflammatory mediators, such as histamine, leukotriene C_4, and vascular endothelial growth factor.

In conclusion, our findings confirm that recurrent angioedema can be associated with persistent peripheral blood eosinophilia. The normalization of the eosinophil count and the disappearance of the angioedema we recorded after anthelminthic treatment suggest that cryptic nematode infestation should be taken into account in patients with concomitant eosinophilia and angioedema in order to choose effective therapy.

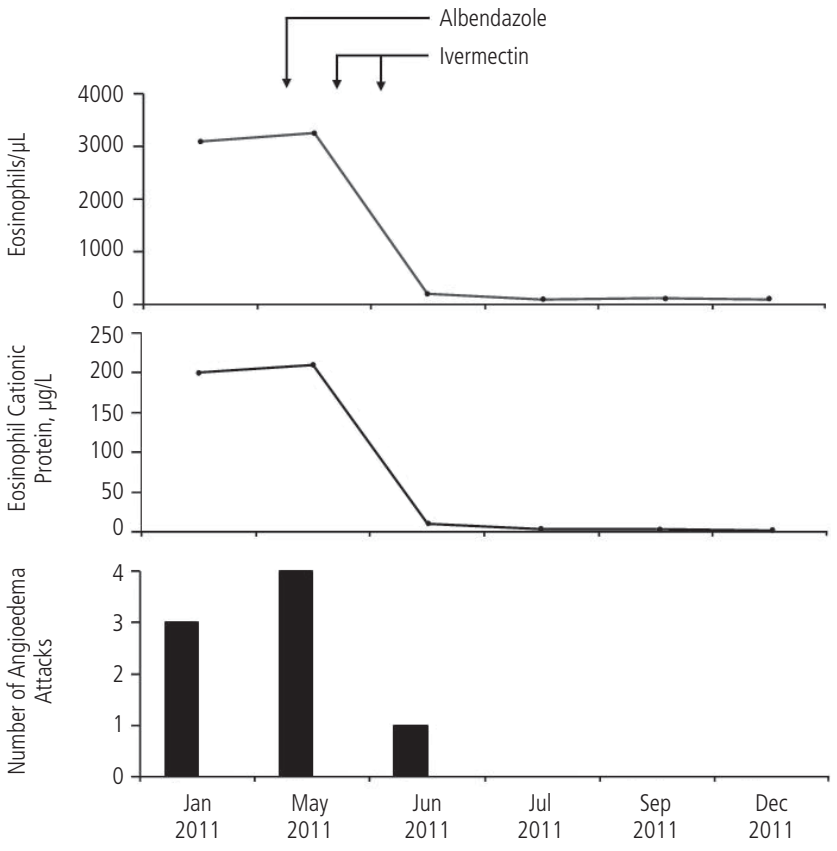

Figure. Effects of anthelminthic treatment with albendazole and ivermectin on the time course of eosinophil counts, serum eosinophil cationic protein levels, and the frequency of angioedema attacks in a 49-year-old man hospitalized for recurrent angioedema and eosinophilia.

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Usefulness of Oral Food Challenge and a Visual Analog Scale in the Etiologic Diagnosis of Eosinophilic Esophagitis

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Palabras clave: Esofagitis eosinofílica. Provocación con alimentos. Escala analógica visual

Eosinophilic esophagitis (EoE) is a chronic inflammatory disorder of the esophagus that is characterized clinically by the presence of symptoms of esophageal dysfunction and histologically by the presence of inflammatory eosinophilic infiltrate in the esophageal mucosa [1].

The pathogenesis of EoE is unknown, but sensitization to foods and aeroallergens has been proposed as a potential cause [2,3]. Skin prick tests (SPTs) and atopy patch tests (APTs) are usually applied to identify causative foods and thus eliminate them from the patient's diet [4].

The definitive evidence that food is causing EoE is based on normalization of biopsy findings after an elimination diet and return of esophageal eosinophils on reintroduction of the food [5]. However, this approach is not always possible, as multiple esophageal biopsies may not be feasible in all patients.

A 42-year-old woman whose diagnosis of EoE was confirmed by histopathology of biopsy specimens taken from the proximal and distal esophagus that showed a dense eosinophilic infiltrate (>20 cells per high-power field) attended our outpatient clinic to ascertain the possible causes of her disease. Other causes of eosinophilic infiltration in the esophagus (eg, gastroesophageal reflux) were ruled out when the results of pH-metry proved to be normal.

The patient had no history of asthma or allergy (specifically no food allergies). For the last 4 years she had experienced dysphagia immediately after eating lentils and egg (most frequently with raw egg). She had no history of food impaction or vomiting and had not previously received swallowed fluticasone propionate or any other symptomatic treatment. At her first visit, she was avoiding lentils and eating egg frequently.

The results of SPTs to a set of aeroallergens and food allergens (milk, egg white, rice, corn, hake, prawn, lentil, pea, peanut, soy, potato, tomato, apple, melon, beef, chicken, and *Anisakis simplex*) could not be measured because of positive dermographism. The APTs were performed as described previously in the literature [4], with 2 g of dry food (skimmed milk powder, dried egg white, wheat, rice flour, corn, potato, hake, beef, chicken, tomato, apple, pea, lentil, and peanut) mixed with 2 mL of isotonic saline solution. Two extra determinations with raw egg (white and yolk) were also performed. The results of the APTs were negative. The peripheral blood eosinophil count was 820/mm^3. Total serum immunoglobulin (Ig) E was 204 IU/mL (UniCAP System); the results for specific IgE to ovalbumin, ovomucoid, lentils, egg yolk, and egg white were negative. Serum eosinophilic cationic protein was 26.8 μg/L (reference value, 20 μg/L).

Although the results of the allergy workup were negative, we recommended an elimination diet excluding egg for at least 8 weeks followed by a double-blind-placebo-controlled food challenge (DBPCFC) to demonstrate tolerance or intolerance to egg, as the patient referred frank dysphagia immediately after eating this food. However, she refused to undergo a DBPCFC with lentils, as she preferred to avoid them.

The patient also refused to undergo biopsy before and after the DBPCFC; therefore, we proposed that she complete a 6-item questionnaire on the symptoms she experienced during the DBPCFC. Each item was measured on a 10-cm visual analog scale (VAS). The patient was asked to place a perpendicular line between the 2 extremes of the VAS to grade symptom severity (0, no symptoms; 10, most severe symptoms).

She was asked to complete the VAS immediately before the DBPCFC, during the DBPCFC (before every dose and every 15 minutes to evaluate symptoms), and 2 hours after the last dose. The items included in the VAS are described in the Table.

Table. Questionnaire Administered for the Visual Analog Scale

1. Do you have difficulty swallowing?
2. Dou you have nausea?
3. Do you feel any retrosternal pain?
4. Do you feel a retrosternal burning sensation?
5. Do you have food impaction?
6. Do you vomit?

The patient underwent an oral DBPCFC with raw egg and cooked egg. Twenty-five minutes after ingesting the third dose of raw egg (8.5 g; cumulative dose, 12 g) she experienced dysphagia, chest pain, meteorism, and hypersalivation. The symptoms increased during the following 30 minutes, as recorded on the VAS, in which the values for the items *Do you have difficulty swallowing?* and *Do you feel any retrosternal pain?* increased from 0 cm (no symptoms) to 2 cm before decreasing over the next 2 hours until they returned to 0 cm. Placebo was well tolerated.

The DBPCFC with cooked egg performed 3 months later was well tolerated, and we observed no difference in the VAS before, during, or after the oral challenge.

The patient is currently avoiding lentils and raw egg and eating cooked egg without symptoms.

EoE is a heterogeneous disorder in which eosinophilic infiltration of the esophagus commonly affects patients who exhibit different food sensitization patterns. IgE-mediated sensitization seems to play an important role in the pathophysiology of EoE, and local esophageal production of IgE has been observed in some patients [6]. However, the absence of sensitization to food does not exclude the possibility of food allergy as a cause of EoE [7]. Although a selective elimination diet based on skin testing (SPT,

APT) has shown promising results in large series in children, with high positive predictive values (>74%) and negative predictive values (88% to 100%) [8], these favorable results have not been reproduced in adult series [9].

In summary, we describe a patient with EoE in whom we were able to identify the causative food by performing a DBPCFC and administering a VAS.

The definitive evidence that a food is causing EoE is provided by a diagnostic biopsy of esophageal tissue. However, this approach is not always possible because of patient refusal. Therefore, we suggest an alternative approach based on DBPCFC and VAS in patients diagnosed with EoE who experience symptoms immediately after ingestion of a specific food. The VAS should be validated before general application in clinical practice.

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Fixed Drug Eruption Caused by Piperacillin-Tazobactam

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Key words: Fixed drug eruption. Piperacillin. Piperacillin-tazobactam. Allergy.

Palabras clave: Exantema fijo medicamentoso. Piperacilina. Piperacilina-tazobactam. Alergia.

Fixed drug eruption (FDE) is an adverse reaction consisting of well-defined edematous macules or plaques that typically recur at the same sites with each administration of a particular agent to which a susceptible patient has become sensitized [1]. Most cases involve sulfonamide antibiotics, tetracyclines, nonsteroidal anti-inflammatory drugs, and, less frequently, penicillins [2,3]. To our knowledge, this is the first case of FDE to piperacillin-tazobactam.

A 69-year-old woman with end-stage renal disease on chronic maintenance hemodialysis via a cuffed tunneled central venous catheter was admitted with pneumonia. She had been admitted repeatedly during the past 3 years for treatment of foot gangrene with superimposed infection. She received multiple antibiotics, including amoxicillin-clavulanic acid, meropenem, vancomycin, and piperacillin-tazobactam, which was administered for 7 days during an admission 1 year earlier. During the current admission, she received a dose of intravenous piperacillin-tazobactam (4.5 g) in the emergency department. Eight hours after the infusion, she developed well-defined, dusky, purple patches (approximately 4 × 5 cm) on the dorsum of both hands, although she did not report any itching or pain with the lesions. Cutaneous lesions were not observed on other parts of the body, and no associated angioedema, respiratory symptoms, trauma, or underlying coagulopathy were detected. The patient was hemodynamically stable. The lesions were considered secondary to a hypersensitivity reaction to piperacillin-tazobactam, and the drug was stopped. The patient was subsequently treated with intravenous cloxacillin (10 days), as pansensitive *Staphylococcus aureus* grew in the peripheral blood culture. Treatment was uneventful. The lesions persisted for 5 days and faded thereafter. The patient was subsequently referred for evaluation of suspected allergy, since she may require piperacillin-tazobactam in the future.

At the checkup, the lesions had completely disappeared. However, the description of the lesions led us to postulate that the patient might have experienced an FDE. The only drugs other than piperacillin-tazobactam administered during admission were paracetamol and chronic treatment for hypertension and ischemic heart disease.

In order to prove that the FDE was due to piperacillin-tazobactam, we performed a graded provocation test with the intravenous formulation diluted in 0.9% normal saline, since piperacillin-tazobactam is not available in an oral formulation. As the therapeutic dose (adjusted for renal failure) was 2.25 g bid, we planned our challenge with 0.5 g (approximately one-quarter of a single dose) on day 1, followed by 2.25 g (a full single dose) on day 2, and 2.25 g administered 12 hours apart (full therapeutic dose) on day 3, with 24 hours between escalations. However, 22 hours after the first dose, the same lesions were observed at the locations initially reported by the admitting physician (Figure). Further doses were not administered. An alert for piperacillin-tazobactam was entered into the patient's electronic record to prevent future administration.

FDE is suspected based on the emergence of characteristic, often recurrent lesions after administration of a drug. Sensitization occurs more readily in patients receiving the causative drugs intermittently rather than in those receiving them continuously [1], as is the case with most antibiotics. While most reactions are limited to characteristic hyperpigmented lesions, some may progress to bullous lesions with subsequent administrations [3]; hence the need for early identification of the culprit drug, which is confirmed using provocation tests.

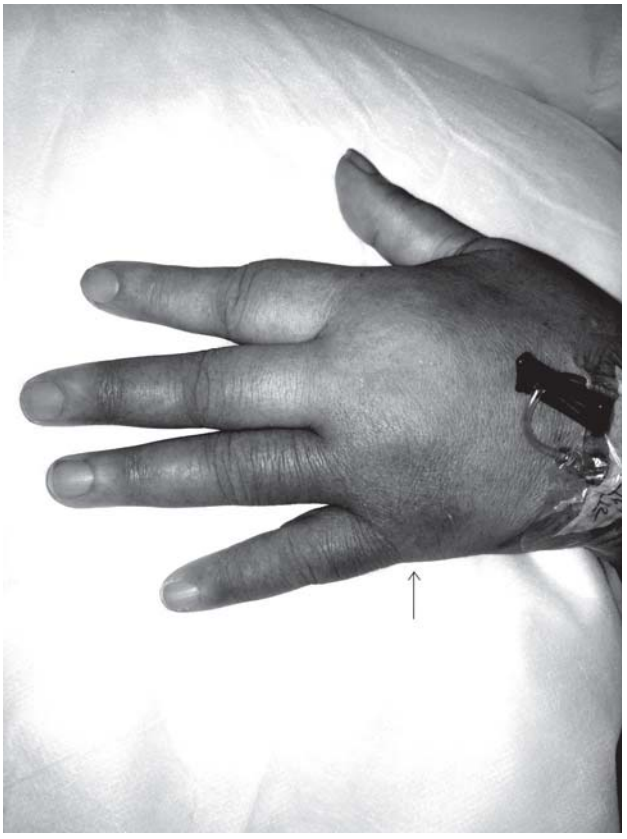

Figure. Fixed drug eruption lesion (arrow) elicited on the dorsum of the patient's hand 22 hours after a provocation test with an intravenous dose (0.5 g) of piperacillin-tazobactam.

Systemic provocation tests are the gold standard for diagnosis of FDE [4]. Topical provocation with patch testing must be performed at the sites of previous lesions, as the results will depend on the activation of intraepidermal $CD8^+$ memory T cells at these sites [2]. While topical provocation tests are safer, the false-negative rate is high. As our patient's rash had completely resolved at the time of her checkup, it was difficult to ensure that the patches were applied at the exact site of the original reaction, thereby further increasing the chance of a false-negative result. Systemic provocation may be required in cases where the reliability of results from a topical provocation test are doubtful [1]. When multiple drugs are implicated, a patch test may serve as a useful screening test.

This case highlights a previously unreported causative agent for FDE. Typical descriptions of a reaction may still lead to the diagnosis of FDE, even after clinical resolution. Identification of the culprit drug is important, especially if the patient is likely to need it in the future. Evaluating for cross-reactivity within the same group of agents may help to define treatment options.

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Profilin May Be a Primary Airborne Sensitizer: A Case Report

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Key words: Respiratory allergy. Profilin. Pollen allergy. Allergens.

Palabras clave: Alergia respiratoria. Profilina. Alergia a polen. Alérgenos.

The plant panallergen profilin, a 12- to 15-kDa actin-binding protein present in all eukaryotic cells, frequently sensitizes patients with pollen allergy and causes both skin reactivity and positive in vitro tests with many different pollen extracts due to its cross-reactive nature [1-3]. Profilin has been shown to be a potentially relevant plant food allergen [4]. However, its importance as an airborne allergen has long been unclear, mainly because sensitization to profilin always accompanies allergy to 1 or more "genuine" pollen allergens, which are considered to induce sensitization to this panallergen [5]. However, 2 recent Spanish studies elegantly showed profilin's potential as an airborne allergen using bronchial and conjunctival tests with purified date palm pollen profilin, Pho d 2 [6,7]. It is generally believed that primary profilin sensitization does not occur. We report a clinical case suggesting that airborne profilin may sometimes behave as a genuine pollen allergen inducing de novo sensitization in genetically predisposed individuals.

A 32-year-old African woman living in Italy for 30 months was seen at the outpatient allergy department of the Clinica San Carlo in Paderno Dugnano at the beginning of July, 2012. The woman reported unremitting rhinitis and conjunctivitis since February 2012 and generalized pruritus following the ingestion of bunch tomatoes from vines grown in southern Italy. Skin prick tests (SPTs) with commercial extracts of the major airborne allergens (Allergopharma) were carried out. An intense skin response to rye grass, plantain, olive, and birch pollen extracts, a weak response to ragweed, mugwort, cypress, pellitory, and plane pollen extracts, and no response to house dust mites, several molds, or cat and dog dander was observed. An SPT with a commercial date palm pollen extract enriched in profilin (50 µg protein/mL, ALK-Abellò) was clearly positive as well.

In order to detect the primary sensitizing pollen source, specific IgE to both genuine pollen allergen components (Phl p 1, Phl p 5, Amb a 1, Art v 1, Cup a 1, Ole 1, Bet v 1, Par j 2) and cross-reacting pollen panallergens (Phl p 7 and Phl p 12 as representatives of calcium-binding proteins and profilin, respectively) was determined by ImmunoCAP (Thermofisher/Phadia). IgE levels were expressed in kU_A/L, and levels exceeding 0.35 kU/L were considered positive. No IgE reactivity to Amb a 1, Art v 1, Phl p 1, Phl p 5, Phl p 7, Bet v 1, Par j 2, or Ole e 1 was detected; Cup a 1, the major cypress allergen, was weakly positive (0.49 kU_A/L), whereas grass profilin was strongly positive (12.6 kU_A/L). In view of these results, in order to exclude primary sensitization to a genuine airborne allergen not included in the list above, specific IgE to further allergen components was detected using the most recent version of the ISAC allergen microarray immunoassay platform (Thermofisher/Phadia), including 112 allergen proteins, according to the manufacturer's recommendations. Reaction sites were incubated with 30 mL of patients' sera for 2 hours. After rinsing, washing, and drying, allergen-specific IgE complexes were stained with a fluorescence-labeled anti-human IgE for 30 minutes. After further washings, a laser scanner took fluorescence readings, and results were transformed into numeric data by comparison with a reference serum standardized against ImmunoCAP IgE. As a consequence, the results, expressed as ISAC standardized units (ISU/L), are indirectly linked to the World Health Organization IRP 75/502 IgE standard. Levels greater than 0.3 ISU/L were regarded as positive.

Weak IgE reactivity was found against the genuine grass pollen allergens Cyn d 1 (0.8 ISU/L) and Phl p 4 (0.4 ISU/L), against Cry j 1 (0.4 ISU/L) and Cup 1 (0.8 ISU/L) from the Cupressaceae group, and against Pla a 2 (0.7 ISU/L) from plane pollen. Moderate to strong IgE reactivity against all profilins included in the panel was found: Bet v 2 from birch (3.2 ISU/L), Hev b 8 from natural rubber latex (16.0 ISU/L), Mer a 1 from *Mercurialis* (7.9 ISU/L), and Phl p 12 from grass (1.6 ISU/L). No IgE reactivity was detected against other genuine allergens from grass (Phl p 1, Phl p 2, Phl p 5, Phl p 6, and Phl p 11), birch (Bet v 1), Olive (Ole e 1, Ole e 9), plane (Pla a 1), ragweed (Amb a 1), mugwort (Art v 1), *Chenopodium* (Che a 1), pellitory (Par j 2), plantain (Pla l 1), *Salsola* (Sal k 1), or natural rubber latex (Hev b 1, Hev b 3, Hev b 5, and Hev b 6). Based on these results, primary sensitization to airborne profilin was diagnosed.

In the case described, profilin acted as a genuine long-lasting airborne allergen that induced primary de novo sensitization. The symptoms started in February during the hazel tree pollen season and were still present in early July, at the end of the grass pollen season. Profilin hypersensitivity was furthermore indirectly confirmed by the appearance of symptoms following the ingestion of raw tomato, a plant-derived food that has been associated with profilin hypersensitivity [4,8]. Skin tests with commercial pollen extracts of pellitory, cypress, ragweed, mugwort, and plane were only weakly positive. While this observation is not surprising for pellitory and cypress [5,9] it is new in the case of ragweed, mugwort, and plane pollen, which are generally strongly positive in profilin-sensitized individuals. This finding might suggest some molecular difference in profilins causing primary or secondary sensitization.

Altogether, this case confirms that profilin may be a relevant airborne allergen [6,7] and shows that in some cases it might actually behave as a genuine airborne allergen.

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Duck Egg Allergy in a Patient Who Tolerates Hen's Eggs

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Key words: Duck egg. IgE-mediated. Food allergy.

Palabras clave: Huevo de pato. Ig E mediada. Alergia alimentaria.

Eggs are among the foods that most frequently cause allergy. Hypersensitivity to dietary substances from egg yolk or white causes an overreaction of the immune system, which can lead to severe physical symptoms for millions of people around the world.

Egg allergy appears mainly, but not exclusively, in children. In fact, it is the second most common food allergy in children, after cows' milk allergy. It is usually treated with an exclusion diet and vigilant avoidance of foods that may be contaminated with egg. The Asthma and Allergy Foundation of America estimates that most children outgrow egg allergy by the age of 5 years, but some people remain allergic for a lifetime [1].

People with allergy to hen's eggs are generally also allergic to eggs from other birds, such as geese or duck [2]. A person with severe egg allergy who is contact-reactive should avoid touching eggs, including wild bird eggs, but it is possible to be allergic to one type of egg but not to others.

We report an unusual case of food allergy in an adult patient after the consumption of duck egg. A 69-year-old man presented with complaints of erythematous itchy papular rash in the perioral area accompanied by swelling of the lips in the area that had been in contact with the egg. The reaction had appeared 20 minutes after eating egg white. The patient also presented breathlessness, wheezing, and skin lesions on his body. Each time the patient had tried duck egg, he presented symptoms, and he had had several episodes over the previous year. The ingestion of hen's eggs did not induce any symptoms. He denied allergy reactions to other foods but did complain of hay fever symptoms in the spring.

Prick-to-prick tests were performed with fresh egg white and yolk from duck eggs and with commercial extract from egg, egg white, egg yolk, ovalbumin (OVA) and ovomucoid (OVM), all from chicken. Specific and total immunoglobulin (Ig) E were measured with the Pharmacia CAP System (Pharmacia Diagnostics), according to the manufacturer's instructions.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and IgE immunoblotting were carried out with egg white extracts from hen (0.75 mg/mL) and duck (0.73 mg/mL). SDS-PAGE was carried out using the Laemmli method [3] with nonreducing conditions and 15% polyacrylamide running gel. The egg white extract proteins separated by SDS-PAGE were transferred onto nitrocellulose membranes as described by Towbin et al [4].

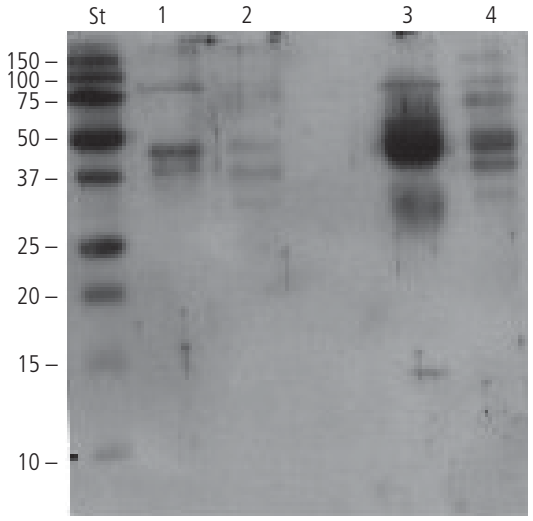

Figure. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel of egg white proteins from hen (lane 1) and duck (lane 3). SDS-PAGE gel of egg yolk proteins from hen (lane 2) and duck (lane 4).

Immunoblotting of IgE-binding protein was achieved by enhanced chemoluminescence according to the manufacturer's instructions (ECL-Amersham). As negative controls, the blots were also incubated with dilution buffer instead of patient serum.

Seventeen patients with mild chicken egg symptoms and a negative response to duck egg were used as controls.

The patient's skin tests to aeroallergens (house dust mites, molds, animal dander, and pollens) were positive to grass pollen and weeds and hen's egg proteins, and negative to egg white (1/20 wt/vol), egg yolk (1/20 wt/vol) (Dome-Hollister-Stier), ovalbumin (OVA) (1 mg/mL), and ovomucoid (OVM) (1 mg/mL) (Leti).

Prick-to-prick tests with fresh uncooked duck egg white and yolk were positive. A double-blind placebo-controlled oral food challenge with duck egg white caused oral pruritus and urticaria after 50 minutes. The patient usually tolerates hen's eggs without symptoms. Immunoblotting (Figure) showed the presence of a band of specific IgE protein with a molecular weight of around 14.4 kDa that might correspond to lysozyme (gal d 4). Only a few previous reports have described allergy to egg white from duck [5].

Egg is one of the most common food allergens affecting young children. The first reaction usually occurs between the ages of 6 and 15 months, when the children are given egg for the first time. Egg allergy is much more common in children than it is in adults, and most individuals overcome their allergy by the time they are 16 years old [6].

The presence of cross-reacting proteins in various avian egg whites has been reported previously. Egg white from different birds (turkey, duck, goose, and seagull, among others) contains proteins that cross-react with most allergens in hen's egg white, but the degree of cross-reactivity varies considerably depending on the egg white. OVA, OVM, and conalbumin from various fowls are all able to bind human IgE but to varying degrees. Duck and goose are classified in the same order (Anseriformes) and the respective egg whites are very similar in terms of immunochemical reactions. According to the results discussed above, it seems reasonable that all egg whites may provoke allergic reactions when ingested by patients who are allergic to hen's egg white. Immunoblotting results indicate that lysozyme might be responsible for the sensitization in the case reported in this paper. Lysozyme from the egg whites tested showed differences in electrophoretic mobility. The antigenic determinant of this protein seems to be specific to Anseriformes (duck and goose), and does not appear to be found in other orders such as Galliformes (hen and turkey) [7].

The considerable variation in specific allergen activity in the various egg whites indicates that patients with hen's egg allergy may tolerate eggs from other birds, for instance, duck or goose [7]. As seen in the current report, our patient tolerated hen's egg despite his allergy to duck and goose egg.

In conclusion, we have described the case of a 69-year-old man without hen's egg allergy who had an IgE-mediated allergy to duck egg white.

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Chronic Granulomatous Disease Caused by a Novel Mutation in a 2-Month-Old Boy With Multifocal Splenic Abscesses

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Key words: Chronic granulomatous disease. Primary immunodeficiencies. Splenic abscesses.

Palabras clave: Enfermedad granulomatosa crónica. Inmunodeficiencia primaria. Abcesos esplénicos.

Chronic granulomatous disease (CGD) is a primary immunodeficiency with an estimated prevalence of 1:250 000 in Europe. It is caused by a defect in the respiratory burst resulting from abnormal functioning of nicotinamide adenine dinucleotide phosphate oxidase (NADPH), a key enzyme in the respiratory burst of phagocytes, which consists of membranous subunits ($gp91^{phox}$, $p22^{phox}$) and cytosolic subunits ($p47^{phox}$, $p67^{phox}$). CGD is caused by a genetically determined defect (autosomal or X-linked) in any of these subunits. The severity of clinical symptoms varies between patients, who are at a higher risk of recurrent infections and formation of granulomas. Diagnosis is confirmed by the absence of respiratory burst in stimulated phagocytes. Genetic testing plays an important role in the confirmation of diagnosis [1].

A 7-week-old boy was referred to our clinic with prolonged fever, increased inflammatory markers, left upper lobe pneumonia, and suspected abscesses in the spleen. His family history was negative for immunodeficiencies and other complicated infectious diseases. His parents were not consanguineous. His BCG vaccination, which was administered according to schedule, was complicated by the formation of a small abscess at the injection site and prolonged healing, although no concomitant lymphadenopathy or other visceral abscesses were detected. During the first month of life, the child experienced an episode of febrile respiratory infection. X-ray imaging revealed left-sided pneumonia, and an otorhinolaryngology examination confirmed bilateral acute serous otitis media. Laboratory tests showed increased inflammatory markers (C-reactive protein, 106 mg/L). One month later, at the age of 6 weeks, he was admitted to the regional hospital owing to another episode of fever, cough, and rhinitis. The laboratory tests showed increased inflammatory markers, and chest x-ray confirmed upper left lobe pneumonia. Cultures from the nose, throat, and urine were negative, and a laryngeal swab sample yielded negative results in polymerase chain reaction for *Mycoplasma, Chlamydophila*, cytomegalovirus, and Epstein-Barr virus. Serology testing for the same pathogens was negative, as was testing for *Aspergillus* and *Candida* antigens. Abdominal ultrasound of the spleen revealed hypoechogenic lesions, and the patient was transferred to our clinic (Figure, A). Magnetic resonance imaging of the spleen showed 15 to 20 microabscesses (15-20 mm) (Figure, B). Given the patient's age, the multifocal involvement of the spleen, and the localization of the abscesses, puncture was not performed. The liver was only slightly enlarged, with no focal changes. Initial antibiotic treatment comprised a combination of ciprofloxacin, vancomycin, and fluconazole administered intravenously for 3 weeks followed by cefixime. The splenic abscesses regressed, and postinflammation calcifications developed (Figure, C). Serum immunoglobulin (Ig) levels were normal (IgG, 4.553 g/L; IgA, 0.187 g/L; IgM, 0.291 g/L; and IgE, 10.2 U/mL), and no deviations in specific cellular immunity or neutropenia were recorded.

Phagocytic activity was normal (61%). The result of the oxidative burst test (dihydrorhodamine flow cytometry) was negative, and chronic granulomatous disease was suspected. The tests performed at the Chronic Granulomatous Disease Diagnosis and Research Centre in Grenoble, France were functional analysis of peripheral mononuclear cells, which revealed no production of superoxide anions with sufficient production in the patient's sister and both parents, and Western blot analysis, which revealed no expression of $p67^{phox}$ protein from the NADPH oxidase complex and decreased concentration of $p40^{phox}$, another protein from this complex. Diagnosis was confirmed with the identification of the causal mutation in the *NCF2* gene encoding $p67^{phox}$, namely, a point mutation (G26→A) in exon 1 leading to the formation of the stop codon

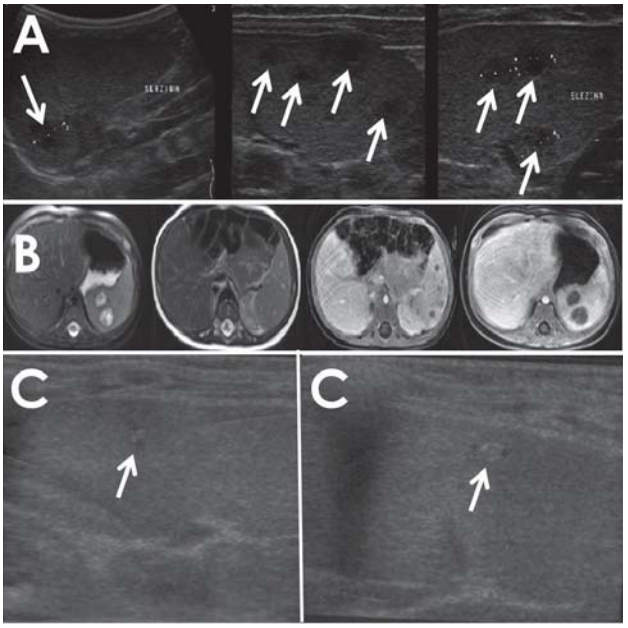

Figure. Multifocal splenic abscesses in a patient with autosomal recessive chronic granulomatous disease. A, Ultrasound findings before treatment. B, Magnetic resonance image. C, Ultrasound findings after treatment, with formation of calcification.

with no expression of the protein. This novel mutation was recently reported by Martel et al [2]. Both parents carried the mutation, although the mutated allele was not detected in the patient's sister. Antimicrobial prophylaxis (cotrimoxazole with itraconazole) was started. The patient's clinical status remains very good at the age of 4 years. None of his relatives can act as HLA-identical donors of hemopoietic stem cells. The frequency of respiratory infections is within the normal range, and other vaccinations on the patient's schedule were well tolerated with no complications. No other signs of skin or visceral abscesses or granulomas have been identified, and splenic findings are stable.

Splenic abscess occurs in patients with specific predisposing factors, such as primary or secondary immunodeficiency, neoplasia, trauma, metastatic infections, splenic infarcts, and diabetes [3]. It is a rare complication in children and remains a diagnostic and therapeutic challenge. Although visceral abscesses are considered to be an important clinical warning sign for primary immunodeficiencies, especially for chronic granulomatous disease, splenic involvement is extremely rare and unusual. The most common sites for abscesses in patients with CGD are the liver, lymph nodes, skin, and perianal region [4]. We found few reports of patients with splenic abscesses as a symptom and consequence of CGD. In 1989, Orduna et al [5] reported 2 cousins with CGD, although the spleen was involved in only 1 of them. Another report described the case of a girl with autosomal recessive CGD presenting as neonatal impetigo, recurrent purulent lymphadenitis, and splenic abscesses [6]. Martel et al [2] recently described 3 patients with splenic abscesses. Splenic abscesses have been reported in both forms of CGD, thus preventing us from concluding that an autosomal recessive form of CGD predisposes patients to the formation of splenic abscesses more than X-linked CGD. Only a further 2 studies of splenic abscesses in CGD in children have been reported [7,8].

The primary pathogens associated with splenic abscesses are *Staphylococcus, Streptococcus, Salmonella, Candida,* and *Aspergillus* species, but other, less common pathogens (*Chromobacterium violaceum, Paecilomyces* species) have been reported [3,9]. *Candida* species is the most common microorganism isolated from patients with multiple splenic abscesses [3]. Treatment consists of combined antimicrobial therapy (antibiotics, antimycotics), although recombinant human granulocyte colony-stimulating factor has also proven successful [10].

In conclusion, although splenic abscesses are a rare finding in clinical practice, they should point to a diagnosis of chronic granulomatous disease, especially in children.

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A Case of DRESS Syndrome Induced by Dipyrone

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Key words: Adverse drug reaction. Dipyrone. Eosinophils.

Palabras clave: Reacción adversa a fármacos. Dipirona. Eosinófilos.

Drug rash with eosinophilia and systemic symptoms (DRESS) syndrome is a rare and severe skin eruption associated with systemic findings such as fever, eosinophilia, lymphadenopathy, and internal organ involvement [1]. The syndrome has been reported to be associated with aromatic anticonvulsants, sulfonamides, allopurinol, and many other drugs. Several mechanisms are involved in its pathophysiology, namely, drug toxicity, immunological imbalance, and viral infections, such as human herpesvirus type 6 (HHV6).

A 32-year-old man was referred to our hospital with liver failure. He had a 1-week history of fever (38.5ºC), diarrhea, jaundice, ascites, hepatomegaly, swollen extremities with pitting edema, and generalized exanthematous pustulosis.

Laboratory tests revealed leukocytosis (12 950/µL) with eosinophilia (3650/µL), abnormal liver and kidney function (alanine aminotransferase [ALT], 1083 IU/L; aspartate aminotransferase [AST], 637 IU/L; bilirubin, 3.2 mg/mL; prothrombin time, 16%; blood urea nitrogen, 75 mg/mL; creatinine, 1.4 mg/dL), hypoproteinemia (2.8 g/dL), and increased nonspecific inflammatory markers (C-reactive protein, lactate dehydrogenase, and erythrocyte sedimentation rate) and eosinophil cationic protein (55 µg/L). The lymphocyte count revealed a predominance of activated $CD8^+$ T cells. Blood and stool cultures were negative, as was serology testing for virus, including HHV6. Radiology and ultrasound examinations showed pericardial and bilateral pleural effusion and confirmed ascites (Figure). The skin biopsy revealed spongiosis and vacuolar degeneration of the epidermal basal keratinocytes. Melanophages, moderate lymphocytic perivascular infiltrate, and intravascular neutrophils were present in the dermis.

Once a diagnosis of DRESS syndrome had been confirmed, methylprednisolone (1 mg/kg/d) was added to the treatment, and both symptoms and laboratory results improved rapidly. The patient was discharged after 7 days with normal blood test results except for mild elevation of aminotransferases (ALT, 121 IU/L; AST, 47 IU/L) and bilirubin (1.9 mg/dL). All parameters returned to normal after a month of treatment with decreasing doses of oral corticosteroids.

The patient reported having undergone surgery to correct an anal fissure 25 days prior to the onset of DRESS syndrome. His postoperative treatment included several oral medications: dipyrone, dimethicone, metoclopramide, oxazepam, *Plantago ovata*, and 2 herbal mixtures containing *Hypericum perforatum* and *Passiflora incarnata* (which he started immediately before the onset of symptoms). He also took some doses of acetaminophen and ibuprofen.

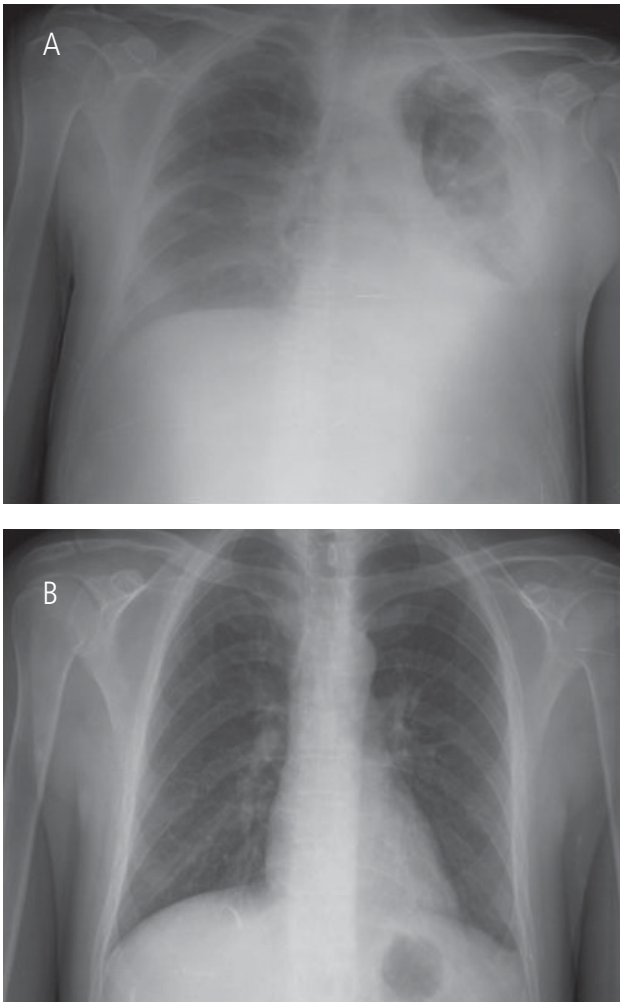

Figure. A, Chest x-ray showing bilateral pleural effusion during an acute episode of DRESS syndrome. B, After resolution.

Although we suspected that the herbal mixture had induced DRESS syndrome, the patient received a long list of forbidden medications including several anti-inflammatory drugs.

When the patient requested safe anti-inflammatory medication at a follow-up visit, we suggested performing a study including skin tests and an oral challenge with anti-inflammatory drugs. He agreed and signed the informed consent. The results of prick and intradermal tests were negative with acetaminophen and dipyrone (readings were performed at 20 minutes, 8 hours, and 1 day after the skin tests). The patient tolerated an oral challenge with acetaminophen 500 mg. The oral challenge with dipyrone 650 mg was initially negative, but the patient was admitted to hospital 12 hours later with arthralgia, myalgia, fever (38.5ºC), choluria, abdominal

pain, and an erythematous rash. Treatment with intravenous methylprednisolone was started, and clinical improvement was evident within 4 hours. Skin rash persisted for 48 hours.

Twelve hours after the oral challenge, the results of blood tests were normal (AST, 22 IU/L; ALT, 19 IU/L), without eosinophilia. Nevertheless, aminotransferase levels had increased at 24 hours (AST, 37 IU/L; ALT 40 IU/L), 48 hours (AST, 43 IU/L; ALT, 65 IU/L), and 72 hours (AST, 29 IU/L; ALT 54, IU/L). Treatment with oral methylprednisolone was maintained for 2 weeks. Ten days after the oral challenge, liver function was normal (AST, 19 IU/L; ALT, 32 IU/L). Leukocyte and eosinophil counts and eosinophil cationic protein levels were normal during this episode.

The patient refused to undergo further evaluations, including patch tests with the herbal mixture and dipyrone.

We report a case of DRESS syndrome associated with several drugs; however, dipyrone was confirmed as the culprit agent by oral challenge. Although challenge tests are contraindicated in DRESS syndrome, we performed them to offer more therapeutic options to a patient with a long list of drug restrictions, including several anti-inflammatory drugs.

We cannot rule out a possible role for the other drugs and herbal mixtures involved in this case. Moreover, it has been suggested that DRESS elicits massive nonspecific activation of the immune system, which decreases the level of tolerance to drugs and facilitates the development of cosensitization to drugs with no chemical or antigenic similarity [2].

The diagnosis of DRESS syndrome is based upon clinical and analytical findings. No well-established tests to identify the culprit agent are available, although patch tests have proven useful in some cases, mostly with antiepileptic drugs [3].

A literature review by Cacoub et al [4] showed that 44 drugs were involved in 172 cases of DRESS syndrome reported between 1997 and 2009. Carbamazepine is the most frequently reported culprit drug [4].

Dipyrone (metamizole sodium) is commonly used in Spain as an anti-inflammatory drug. It can cause severe but rare adverse reactions such as anaphylactic shock [5] and Stevens-Johnson syndrome [6]

DRESS syndrome associated with other anti-inflammatory drugs such as piroxicam [7], ibuprofen [8], and celecoxib [9] has rarely been reported. To our knowledge, this is the first case of DRESS syndrome induced by dipyrone.

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