

Successful Desensitization to Mycophenolate Mofetil: A Case Report

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Systemic lupus erythematosus (SLE) is an autoimmune connective tissue disorder with a wide range of clinical features. Diagnosis is based on clinical assessment supported by investigations, including autoantibody determinations [1]. In a significant number of patients, SLE can cause nephritis, which can result in renal failure, with significant morbidity and mortality [2]. The immunosuppressive agents commonly used to treat SLE (eg, corticosteroids, cyclophosphamide, azathioprine, and ciclosporin) have a high incidence of severe side effects [3]. Consequently, mycophenolate mofetil (MMF) was used to treat lupus nephritis, initially in uncontrolled cohort studies and then in randomized controlled trials [2].

Hypersensitivity to MMF in SLE and successful desensitization to MMF were first reported by Szyper-Kravitz et al in 2005 [4]. The clinical picture comprised a maculopapular rash that was suggestive of type IV hypersensitivity reaction.

We report the case of a patient with SLE that was refractory to standard immunosuppressive regimens who developed a hypersensitivity reaction to MMF and underwent successful desensitization. The patient experienced maculopapular rash with the first dose and anaphylaxis with the second, thus indicating both type I and type IV hypersensitivity reaction. No reactions occurred during the desensitization procedure, and the final total dose of 2000 mg was successfully administered.

A 24-year-old woman diagnosed with SLE at the age of 19 was referred to our allergy outpatient clinic because of previous hypersensitivity reactions to MMF. The patient had been receiving corticosteroids for 5 years. Azathioprine was included in the treatment schedule to control SLE but was suspended because of its side effects. Since the disease remained uncontrolled with conventional therapy, MMF was started at a daily dose of 2000 mg. A maculopapular rash developed during the first 2 weeks, and treatment was stopped. The reaction was successfully managed with methylprednisone and pheniramine, and the rash resolved within 3 days. Three weeks later her doctors decided to reintroduce MMF to the

treatment schedule. Thirty minutes after taking 1000 mg of MMF orally, she experienced an anaphylactic reaction, which manifested as dyspnea, palpitations, and hypotension, and was immediately given 0.3 mg of epinephrine, 45 mg of pheniramine, and 40 mg of methylprednisolone. The reaction resolved within 2 hours.

A skin test with the culprit drug was performed to investigate drug hypersensitivity. This test was conducted 4 weeks after the last reaction to minimize the likelihood of a false-negative result.

A skin prick test was performed on the volar forearm with the drug crushed and diluted in 1 mL of 0.9% saline solution according to a method described elsewhere [5]. The result was negative after 15 minutes. Since intradermal skin testing was unviable because a parenteral form of the drug does not exist, a patch test was performed with the drug crushed and diluted in petrolatum (30% drug + 70% petrolatum) [5]. This test revealed a positive reaction (++) in the first hour. Vesicles and papules appeared at the patch test site on the second day. The positivity in the first hour was considered an irritant reaction, but the positivity on the second day was considered a delayed-type reaction. Skin testing in 10 healthy volunteers did not reveal a positive reaction, thus confirming the positivity of the patch test result.

As alternative immunosuppressive treatment was not recommended, a desensitization protocol with MMF was planned. After the patient gave her informed consent, desensitization was carried out in an intensive care setting with oral doses of MMF that increased according to the schedule presented in the Table; 40 mg methylprednisone and 45.5 mg pheniramine were administered intravenously 30 minutes before initiation of the protocol. No adverse reactions were observed during the desensitization procedure, and the patient eventually tolerated a total dose of 2000 mg.

We report the case of a patient with a history of anaphylaxis and maculopapular rash due to MMF to treat SLE. The patient successfully completed a desensitization protocol for MMF.

The literature contains only 1 report of a patient with SLE who experienced a maculopapular rash due to MMF and was successfully desensitized [4]. Szyper-Kravitz et al [4] administered the desensitization protocol based on gradual

Table. Protocol for MMF Desensitization

Time	Day 1	Day 2	Day 3
8:00 AM	25 mg	75 mg	300 mg
2:00 PM	25 mg	75 mg	600 mg
8:00 PM	50 mg	150 mg	1100 mg
2:00 AM	50 mg	150 mg	
Total dose	150 mg	450 mg	2000 mg

dosing of MMF in an attempt to reach a daily target dose of 1000 mg in 3 days [4]. As the patient we report had a history of anaphylaxis with MMF, our desensitization protocol was performed over 3 days with a lower initial dose followed by increasing doses to reach the therapeutically effective dose of 2000 mg. In our protocol, the dosing intervals were set at 6 hours, taking into consideration the previous delayed-type hypersensitivity reaction and the mean elimination half-life of the active metabolite. No complaints or complications were recorded during the process. Desensitization was completed successfully at the end of the third day.

The anaphylactic reaction in the clinical history points to a type I hypersensitivity reaction, while the maculopapular rash and delayed positive patch test result are indicative of a type IV hypersensitivity reaction. The absence of a maculopapular rash with the second dose can probably be explained by the quick treatment of the anaphylactic episode. The positive result in the patch test during the first hour was probably an irritant reaction, although neither mechanism could be confirmed with *in vitro* tests. Nevertheless, our desensitization protocol seems to be effective in both type I and type IV hypersensitivity reactions. The diagnostic tools used for this patient had not been previously validated; therefore, we cannot estimate their sensitivity and positive predictive values. Furthermore, we did not perform a controlled challenge with the culprit drug, as this would have been very dangerous for this patient.

In conclusion, we present a successful desensitization protocol for both an immediate-type and a delayed-type hypersensitivity reaction due to MMF. Our experience highlights the importance of desensitization in patients where no alternative therapies are available for similar chronic diseases.

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References

1. D'Cruz DP, Khamashta MA, Hughes GR. Systemic lupus erythematosus. *Lancet*. 2007 Feb 17;369(9561):587-96.
2. Yong P, D'Cruz DP. Mycophenolate mofetil in the treatment of lupus nephritis. *Biologics*. 2008;2(2):297-310.
3. Karim MY, Alba P, Cuadrado MJ, Abbs IC, D' Cruz DP, Khamashta MA, Hughes GR. Mycophenolate mofetil for systemic lupus erythematosus refractory to other immunosuppressive agents. *Rheumatology*. 2002;41:876-82.
4. Szyper-Kravitz M, Sheinberg P, Sidi, Schiffenbauer Y, Trubniykov E, Shoenfeld Y. Hypersensitivity to mycophenolate mofetil in systemic lupus erythematosus: diagnostic measures and successful desensitization. *Int Arch Allergy Immunol*. 2005;138:334-6.
5. Brockow K, Garvey L, Aberer W, Atanaskovic-Markovic M, Barbaud A, Biló MB, Bircher A, Blanca M, Bonadonna B, Campi P, Castro E, Cernadas JR, Chiriac AM, Demoly P, Grosber M, Gooi J, Lombardo C, Mertes PM, Mosbech H, Nasser S, Pagani M, Ring J, Romano A, Scherer K, Schnyder B, Testi S, Torres M, Trautmann A. Skin test concentrations for systemically administered drugs – an ENDA/EAACI Drug Allergy Interest Group position paper *Allergy*. 2013;68(6):702-12.

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Delayed Hypersensitivity to Ribavirin Confirmed by Provocation Test

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Key words: Delayed hypersensitivity reaction. Hepatitis C. Ribavirin. Provocation test.

Palabras clave: Reacción retardada de hipersensibilidad. Hepatitis C. Ribavirina. Prueba de provocación.

Infection by hepatitis C virus (HCV) is one of the leading causes of chronic liver disease in the world [1]. HCV infection leads to dermatological and mucocutaneous manifestations, including small-vessel vasculitis as part of mixed cryoglobulinemia syndrome [2]. Chronic HCV infection is treated using the combination of pegylated interferon and ribavirin, which is associated with well-characterized dermatological adverse events [2]. Sometimes, the reactions are so severe that they can interfere with the patient's quality of life and lead to transient or definitive interruption of therapy [3]. The most common side effect (60% of patients) is localized inflammatory skin lesions at the injection site [4]. Other side effects include psoriasis, eczematous reactions, alopecia, sarcoidosis, lupus, fixed drug eruption, pigmentation disorders, and lichenoid eruption [5]. Pegylated interferon and ribavirin have both been associated with hypersensitivity reactions, although the underlying pathophysiology of these reactions is not completely understood [1]. Most of the cutaneous side effects of ribavirin have been reported in patients receiving it in combination with pegylated interferon, thus making it difficult to establish a direct causal relationship [1].

We present a case of a hypersensitivity reaction to ribavirin confirmed by a positive provocation test.

The patient was a 45-year-old white female with a history of chronic hepatitis C who was receiving pegylated interferon alfa-2a combined with ribavirin. Four months after starting therapy she developed generalized pruritic erythematous maculopapular lesions. No other symptoms (respiratory, gastrointestinal, or cardiovascular) were observed. She denied fever, arthritis, asthenia, or any other constitutional complaints. The cutaneous lesions disappeared spontaneously within 15 days of discontinuation. Once the gastroenterologist gave the indication to restart therapy, she was referred to our Drug Allergy Unit.

We performed skin prick tests (SPT) with pegylated interferon alfa-2a at a concentration of 360 µg/mL and intradermal tests (IDT) at 1:1000 to 1:10 dilutions in saline. The results were negative (immediate and late readings). Saline solution and histamine phosphate (10 mg/mL) were used as negative and positive controls, respectively. SPT results were considered positive if the mean wheal diameter was ≥ 3 mm greater than the negative control. IDT results

were considered positive if there was a 2-fold increase in the mean wheal diameter. We also performed patch tests with pegylated interferon alfa-2a diluted at 1:100 to 1:1 and with different concentrations of ribavirin (10%, 30%, and 50% in petrolatum); the results were negative. Saline solution and petrolatum were used as negative controls. Patch tests were scored according to the criteria of the International Contact Dermatitis Research Group [6]. Subcutaneous challenge with a cumulative dose of 180 µg of pegylated interferon alfa-2a did not cause an immediate or delayed reaction. A single-blind placebo-controlled oral provocation test with increasing doses of ribavirin up to 1000 mg did not cause an immediate reaction. However, 6 hours after provocation, the patient developed generalized pruritic erythematous micropapular lesions, which progressed to confluent lesions with infiltration, on the arms and thighs and erythema on the lower back. No other symptoms were observed. The skin lesions resolved in 3 days with oral antihistamines and corticosteroids. A skin biopsy was not performed. The lymphocyte transformation test was performed with ribavirin 100 µg/mL and the result was doubtful, with a stimulation index of 2.7 (positive >3). The patient was advised to avoid ribavirin. The gastroenterologist reported that monotherapy was not indicated, since there was no significant progression of liver disease.

Interferon and ribavirin are usually suspected in hypersensitivity reactions when symptoms resolve rapidly after discontinuation. The mechanism by which this combined treatment causes cutaneous hypersensitivity reactions has not been elucidated [1]. Consequently, skin tests with those drugs are seldom performed, as they are mostly unhelpful [7,8]. In the present case, the results of patch tests with ribavirin were negative, and those of the lymphocyte transformation test doubtful, possibly owing to the lack of standardization and the low sensitivity of skin tests for delayed hypersensitivity reactions. In the case of the lymphocyte transformation test, the formulation used was not the most appropriate (tablets).

Most of the cutaneous side effects of ribavirin have been reported in patients receiving the drug in combination with pegylated interferon, thus making it difficult to establish a direct causal relationship [1]. The literature we reviewed indicated that reactions during combination therapy are caused by pegylated interferon, although the involvement of ribavirin is not excluded [9]. A recent article describes a case of successful desensitization to ribavirin, even though a hypersensitivity mechanism was not demonstrated [1]. The authors emphasize the need for provocation tests with both drugs because the culprit drug can be ribavirin, as in the present case. To our knowledge, we report the first case of hypersensitivity reaction to ribavirin confirmed by a positive provocation test.

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References

1. Ladd A, Martel-Laferriere V, Dieterich D. Successful Desensitization to Ribavirin in a Patient With Chronic Hepatitis C. *J Clin Gastroenterol*. 2012;46:716-7.
2. Cacoub P, Bourlière M, Lübke J, Dupin N, Buggisch P, Dusheiko G, Hézode C, Picard O, Pujol R, Segaert S, Thio B, Roujeau JC. Dermatological side effects of hepatitis C and its treatment: patient management in the era of direct-acting antivirals. *J Hepatol*. 2012;56:455-63.
3. Nogueira J, Sena L, Quintans J, Almeida J, França A, Júnior L. Side effects of the therapy with peginterferon and ribavirin in chronic hepatitis C: a small audit. *J Pharm Pract*. 2012;25:85-8.
4. Michael W. Side effects of therapy of hepatitis C and their management. *Hepatology*. 2002;36:S237-44.
5. Mistry N, Shapero J, Crawford R. A review of adverse cutaneous drug reactions resulting from the use of interferon and ribavirin. *Can J Gastroenterol*. 2009;23:677-83.
6. Beltrani V, Bernstein I, Cohen D, Fonacier L. Contact dermatitis: a practice parameter. *Ann Allergy Asthma Immunol*. 2006;97:S1-38.
7. Savk E, Uslu G, Karaoglu AO, Sendur N, Karamant G. Diffuse cutaneous eruptions due to interferon alfa and ribavirin treatment of chronic hepatitis C. *J Eur Acad Dermatol Venereol*. 2005;19:396-8.
8. Dereure O, Raison-Peyron N, Larrey D, Blanc F, Guilhou J. Diffuse inflammatory lesions in patients treated with interferon alfa and ribavirin for hepatitis C: a series of 20 patients. *Br J Dermatol*. 2002;147:1142-6.
9. Hashimoto Y, Kanto H, Itoh M. Adverse skin reactions due to pegylated interferon alpha 2b plus ribavirin combination therapy in a patient with chronic hepatitis C virus. *J Dermatology*. 2007;34:577-82.

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Selective IgM Deficiency in a Boy With Ring Chromosome 18

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Key words: Selective IgM deficiency. Ring chromosome 18. Recurrent respiratory tract infections.

Palabras clave: Deficiencia selectiva de IgM. Anillo cromosoma 18. Infecciones recurrentes del tracto respiratorio.

The incidence of chromosome 18 deletion syndrome is about 1 per 40 000 live births, and 18q- is one of the most common autosomal deletion syndromes in humans [1]. The syndrome is characterized by mental retardation, hypotonia, typical facial dysmorphism, foot deformity, short stature, long tapering fingers, microcephaly, and multisystemic abnormalities including cardiac, endocrine, genitourinary, immunologic, and neurologic manifestations [2].

The association between immunodeficiency and chromosome 18 abnormalities has been widely reported, and most cases generally involve selective IgA deficiency and agammaglobulinemia [3,4]. In this report, we present the case of a boy with de novo ring chromosome 18 and selective IgM deficiency. To our knowledge, this is the first case report describing the association between ring chromosome 18 and selective IgM deficiency.

A 3-year-old Turkish boy with a known chromosomal abnormality was referred to the pediatric immunology outpatient clinic because of recurrent respiratory tract infections. He was born at term after an uneventful gestation (birth weight, 3500 g). His parents were healthy and nonconsanguineous. There was no family history of immunodeficiency or other hereditary diseases. The patient is being followed-up in a pediatric genetics clinic because of de novo mosaic ring chromosome 18, karyotype mos 46,XY,r(18)(p11.2q23)[97]/45,XY,-18[3] (Figure). The parental karyotypes were normal. He has experienced recurrent lower and upper respiratory tract infections for more than 2 years. At the age of 2 years, adenotonsillectomy was performed because of recurrent otitis media. His history also included failure to thrive and delayed developmental milestones. Cranial magnetic resonance imaging revealed corpus callosum hypoplasia and delayed myelination; echocardiography revealed subaortic ventricular septal defect and secundum atrial septal defect. The physical examination disclosed short stature, hypotonia, strabismus, and dysmorphic features including microcephaly,

Immunoglobulin Levels	3 y	Normal Range	6.5 y	Normal Range
IgM, mg/dL	11	43-207	11	43-207
IgA, mg/dL	51	14-159	86	33-236
IgE, IU/mL	96	0-230	28	0-230
IgG, mg/dL	390	345-1236	841	608-1572
IgG1	314	310-729	573	380-866
IgG2	62	46-387	206	106-481
IgG3	23	10-96	28	14-115
IgG4	6	1-77	8	1-115
Anti-A isohemagglutinin titers	1:1	>1:10	1:4	>1:10
Lymphocyte subsets, %				
CD3	63	43-76	-	-
CD4	37	23-48	-	-
CD8	26	14-33	-	-
CD19	29	14-44	-	-
CD16-56	8	4-23	-	-

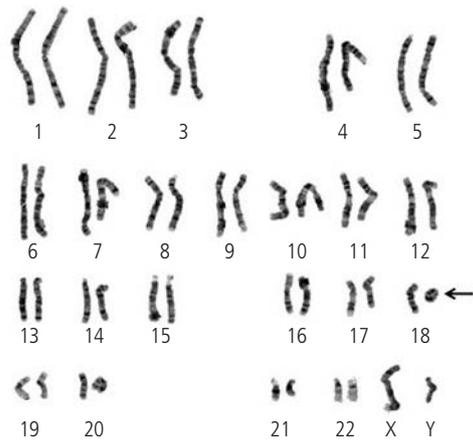


Figure. A, Immunological work-up of the patient over time. B, Patient's karyotype analysis by G-banding technique. The arrow indicates ring chromosome 18.

bilateral epicanthus, hypertelorism, low-set ears, micrognathia, small teeth, short neck, clinodactyly, and micropenis.

Baseline immunological investigations revealed normal white blood cell and platelet counts. The absolute neutrophil and lymphocyte count were also within normal limits. Serum levels of IgA, IgE, IgG, and IgG subgroups were normal except for IgM, which was constantly below the normal range at subsequent visits (Figure). His blood type was B (Rh+), and the anti-A isohemagglutinin titer was 1:1 ($N > 1:10$). The results of flow cytometry for lymphocyte subsets and the lymphocyte proliferation test with phytohemagglutinin were

within normal limits. Based on these laboratory findings, a diagnosis of selective IgM deficiency was confirmed. Lower respiratory infections resolved after intravenous immunoglobulin (IVIG) replacement therapy was initiated (400 mg/kg/dose, every 3 weeks).

At his last examination at age 6.5 years, he was free of lower respiratory tract infections under IVIG therapy but had short stature, morbid obesity, and micropenis. His height was 108 cm (SD, -1.95), his weight was 31 kg, and his body mass index was 28.7 (SD, $+2.91$). Penis stretch length was 3 cm. Endocrinological investigations did not reveal any pathologic conditions. Lipid profile, glucose/insulin ratio, thyroid function tests, cortisol level, and IGF-1 level were all within normal limits. Ultrasound images of the kidneys and liver were normal. In the last immunological study, serum IgM level, and anti-A isohemagglutinin titer were found to be low (Figure).

Selective IgM deficiency is a rare disorder, with a reported prevalence of 0.03%. It is characterized by isolated low levels of serum IgM with no other identifiable immunodeficiency. Serum IgM levels are usually < 20 mg/dL in infants and children or < 2 SD or 10% below age-adjusted means [5]. Selective IgM deficiency is a heterogeneous disorder with no known genetic component and may occur as a primary or a secondary condition. The disease can be complicated by bacterial, viral, fungal, and parasitic infections, which usually result in recurrent respiratory tract infections such as otitis media, chronic rhinosinusitis, bronchitis, and pneumonia.

Management of patients with selective IgM deficiency includes antibiotic prophylaxis and IVIG therapy in the case of recurrent infections or specific antibody deficiency. Yel et al [6] described 5 patients with recurrent, severe infections and selective IgM deficiency who responded very well to IVIG therapy and concluded that IgM-deficient patients who present with recurrent, severe infections could benefit from treatment with immunoglobulin, particularly in the presence of impaired pneumococcal antibody responses. In the case we report, even though we could not measure specific antibody response, the patient was treated with IVIG (400 mg/kg every 3 weeks), to which he responded very well.

Ring chromosome 18 is a disorder in which one or both ends of chromosome 18 are lost and the ends join to form a ring. Patients with ring chromosome 18 can therefore show features of 18q- syndrome, 18p- syndrome, or a combination of both depending on the size of the 18p and 18q deleted regions. The association between immunodeficiency and chromosome 18 abnormalities has been widely reported, and most authors have generally focused on selective IgA deficiency and agammaglobulinemia [3,4]. Furthermore, it has been reported that chromosome 18p deletion and IgA deficiency can also be associated with specific polysaccharide antibody deficiency [7]. Dostal et al [8] identified a potential susceptibility gene locus for IgA deficiency in patients with 18q deletion syndrome at a distal region of 18q22.3-q23. This result is in line with the finding of Lewkonja et al [9], who demonstrated the association between IgA deficiency and chromosome breakage at 18q23. Li et al [10] suggested that the nuclear factor of activated T-cell gene, which is located at the q-terminus of chromosome 18, might be responsible

for the association between 18q deletion and IgA deficiency. However, no such findings have been reported for selective IgM deficiency and 18q deletion syndrome. The association of ring chromosome and Ig abnormalities suggests the presence of an as yet unrecognized gene with a pivotal role in Ig production on chromosome 18.

In conclusion, we report for the first time the association between ring chromosome 18 and selective IgM deficiency. Our findings indicate that patients with chromosome 18 abnormalities should also be investigated for selective IgM deficiency, especially if there is a history of recurrent respiratory tract infection.

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Conflicts of Interest

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References

1. Cody JD, Pierce JF, Brkanac Z, Plaetke R, Ghidoni PD, Kaye CI, Leach RJ. Preferential loss of the paternal alleles in the 18q-syndrome. *Am J Med Genet.* 1997;69:280-6.
2. Wertelecki W, Gerald PS. Clinical and chromosomal studies of the 18q- syndrome. *J Pediatr.* 1971;78:44-52.
3. Litzman J, Brysova V, Gaillyova R, Thon V, Pijackova A, Michalova K, Zemanová Z, Lokaj J. Agammaglobulinaemia in a girl with a mosaic of ring 18 chromosome. *J Paediatr Child Health.* 1998;34:92-4.
4. Slyper AH, Pietryga D. Conversion of selective IgA deficiency to common variable immunodeficiency in an adolescent female with 18q deletion syndrome. *Eur J Pediatr.* 1997;156:155-6.
5. Cassidy JT, Nordby GL. Human serum immunoglobulin concentrations: prevalence of immunoglobulin deficiencies. *J Allergy Clin Immunol.* 1975;55:35-48.
6. Yel L, Ramanuja S, Gupta S. Clinical and immunological features in IgM deficiency. *Int Arch Allergy Immunol.* 2009;150:291-8.
7. Browning MJ. Specific polysaccharide antibody deficiency in chromosome 18p deletion syndrome and immunoglobulin A deficiency. *J Investig Allergol Clin Immunol.* 2010;20:263-6.
8. Dostal A, Linnankivi T, Somer M, Kahkonen M, Litzman J, Tienari P. Mapping susceptibility gene locus for IgA deficiency at del(18)(q22.3-q23); report of familial cryptic chromosome t(18q; 10p) translocations. *Int J Immunogenet.* 2007;34:143-47.
9. Lewkonia RM, Lin CC, Haslam RH. Selective IgA deficiency with 18q+ and 18q- karyotypic anomalies. *J Med Genet.* 1980;17:453-6.
10. Li X, Ho SN, Luna J, Giacalone J, Thomas DJ, Timmerman LA, Crabtree GR, Francke U. Cloning and chromosomal localization of the human and murine genes for the T-cell transcription factors NFATc and NFATp. *Cytogenet Cell Genet.* 1995;68:185-91.

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Improving Care of Hereditary Angioedema With C1 Inhibitor Deficiency (Type 1 and Type 2 Hereditary Angioedema) in Latin America

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Palabras clave: Angioedema hereditario. C1 inhibidor. C1 esterasa inhibidor. Angioedema. Latinoamérica.

Hereditary angioedema (HAE) is caused by C1 inhibitor deficiency (HAE-C1INH-D). It is a rare autosomal dominant disease with an estimated prevalence of 1:50 000 [1]. Based on this prevalence, at least 11 000 patients are thought to be affected in Latin America (569 million inhabitants).

Despite recent advances in and greater knowledge of this disease, access to laboratory tests and therapy is limited in most parts of Latin America. The objective of this report is to summarize the available tests and therapeutic interventions in Latin America.

Physicians with expertise in caring for patients with HAE-C1INH-D from Panama, Chile, Peru, El Salvador, Costa Rica, Colombia, Mexico, Brazil, Uruguay, and Argentina met in 2012 to determine the barriers that inhibit optimal care and how to remove these barriers in order to improve care of the patient with HAE-C1INH-D. The objective of the meeting was to draft a white paper to improve diagnosis and care of patients.

The process was initiated in Brazil, where a survey on HAE-C1INH-D was developed and forwarded to the main centers for treatment of patients with HAE-C1INH-D in Latin America.

The study sample comprised 500 patients from cohorts in Argentina, Brazil, and Mexico [2-4]. This number recently grew to 575 as a result of efforts to develop a Latin America-wide database (Table), although it remained far below the anticipated prevalence (5.2% out of 11 000).

Our data suggest that lack of testing resulted in the failure to identify most patients. For example, 10 cases were identified in Costa Rica through tests performed in the USA. Only 2 cases each were identified in Chile, El Salvador, Panama, and Peru, and only 1 in Uruguay. These findings suggest that access to laboratory testing is the first step necessary for identification of HAE-C1INH-D in Latin America.

Even without diagnostic tests, many patients can be diagnosed based on a comprehensive history and family history; however, 25% of cases are new mutations and lack a family history [5,6]. The clinical history helped to identify most cases in Brazil (71.4%), Argentina, Mexico (61.7%), and Colombia (40%).

The first symptoms of HAE-C1INH-D, age of diagnosis, and mean time to diagnosis (6 to 13 years) were similar to those reported elsewhere [2-4,7]; however, the age at diagnosis varied significantly in our cohort (Table).

One of the findings that deviated most from published data was involvement of the upper airway. Previous reports, mainly from Europe, suggest upper airway swelling is expected to develop in 30%-56% of untreated patients during their lifetime [1,7]; however, our data suggest that only 7%-31% of patients have edema of the upper airway.

The survey not only generated significant information about patients with HAE-C1INH-D, but it also acted as a teaching instrument, heightened awareness, and stimulated discussion, leading to increased numbers of patients being evaluated and diagnosed.

The main concern throughout Latin America is poor access to laboratory tests. Since C4 levels are usually reduced in >95% of patients, C4 testing should be encouraged, considering that C1-INH tests are rarely available in Latin America [1,2,8]. In Costa Rica and El Salvador, patients were diagnosed based on tests performed in US laboratories; however, the cost is rather prohibitive (\$200-250 per test).

Traditional drugs for prophylaxis of HAE-C1INH-D include C1 inhibitors, androgens (danazol and oxandrolone), and antifibrinolytics (tranexamic acid and ϵ -aminocaproic acid). Danazol is available throughout Latin America, while oxandrolone is only available in Brazil. A large percentage of patients in Latin America receive danazol for prophylaxis (60%). Tranexamic acid is available in all countries except Costa Rica and Panama.

Table. Reports of Cases of HAE-C1INH-D From Latin American Countries^a

	Argentina ^b	Argentina ^c	Argentina ^d	Brazil ^e	Brazil ^f	Mexico ^g	Colombia	Costa Rica	Peru	El Salvador
Family history, No.	207	6	11	210	59	34	21	10	2	2
Gender, male/female	34/62	2/4	4/7	77/133	22/37	6/28	6/15	5/5	1/1	1/1
Age	Range, 1-70 y	Range, 1.5-33 y	Mean (range) 14 (6-48)	Mean (SD), range 30 (17) y, 1-89, N=199	28 y	Mean (range) 32 (18-72) y, 29 adults; 10 (8-14) y, 5 children	<10 y, 3/21; 10-20 y, 1/21; >20 y 16/21	Range, 8 -53 y	30 y and 48 y	22 y and 19 y
First symptoms	No data	4.3 y	Mean (range) 30 mo (12-96)	Mean (SD) 11.1 (11.8) y, 0.08-64 N=139	12 y	No data	No data	No data	13 y and 1 y	3 y and 14 y
Age at diagnosis	29 y	10.2 y	15.3 y	Mean (SD) 21 (14) y, 0.5-70 N=150	25	Mean (range) 20 (4-56) y, 29 adults; 4 (2-14) y, 5 children	No data	No data	1 y (both)	24 y and 22 y
HAE I, No.	196 (94.7%)	6	11	205	58	34	20	10	1	2
HAE II, No.	11 (5.3%)	0	0	5	1	0	1	0	1	0
Clinical manifestations	Edema: facial (23%), genitalia (6%), limbs (12%), abdomen (60%), larynx (7%)	Facial edema (4/6), laryngeal edema (1/6), abdominal pain (1/6)	Facial and limb edema (9/11), laryngeal edema (1/11), abdominal pain (8/11), asymptomatic (1/11)	SC edema, 170 (80.9%); GI attacks, 114 (54.3%); laryngeal edema 45 (21.4)	SC edema in extremities and face; abdominal pain; dermographism, pressure urticaria (1/66)	GI attacks 34; laryngeal edema 31	No data	SC edema: face, extremities 7/10, laryngeal edema 1/10	SC edema: face 2/2, genitals 1/2, extremities 2/2	AE face 2/2, AE extremities 1/2, laryngeal edema 2/2
Diagnostic delay	No data	6 y	12.7 y	11 y	13 y	No data	No data	No data	1 y	22 y and 8 y

Abbreviations: AE, angioedema; GI, gastrointestinal; HAE, hereditary angioedema; SC, subcutaneous.

^aChile and Panama have 2 cases each and Uruguay has 1 case; these data were not included in the table.

^bInstituto Argentino de Alergia e Inmunología, Buenos Aires, Argentina and Fabiani et al, 2000 [3].

^cServicio de Inmunología del Hospital Pediátrico Prof. J P Garrahan, Buenos Aires, Argentina.

^dServicio de Inmunología del Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina.

^eFirst Brazilian Registry including patients from Rio de Janeiro and Grumach et al 2012 [4].

^fServiço de Imunologia do Hospital Universitário Clementino Fraga Filho da Universidade Federal do Rio de Janeiro.

^gInstituto Nacional de Pediatría, Unidad de Genética de la Nutrición, México DF and Majluf-Cruz and Nieto-Martínez S, 2011

The development of new specific therapies played a key role in improving the quality of life of patients in North America and Europe, although the impact in Latin America has been less than optimal [9]. Plasma-derived C1 inhibitor, which was introduced more than 30 years ago in Argentina, has recently been approved in Brazil and Mexico only. Icatibant was introduced in Brazil, Colombia, Mexico and Argentina, although uptake has been slow because of cost. Ecallantide and recombinant C1 inhibitor are not yet available in Latin America.

Most countries in Latin America still use fresh frozen plasma as rescue therapy for attacks; however, the World Allergy Organization suggests that once specific therapies are available, use of fresh frozen plasma should be limited [10].

Multiple barriers exist and need to be removed to improve diagnosis and treatment of HAE in Latin America. For example, HAE-C1INH-D registries should be developed throughout the region, and new educational initiatives should be undertaken to improve knowledge about HAE-C1INH-D. Similarly, collaborative activities should be developed among patients, patient groups, and physicians. Access to laboratory tests is essential; therefore, it is necessary to develop centers with laboratories and appropriate treatments. New drugs must be approved more quickly, and access to prophylaxis should be facilitated. Finally, in order to explore the best options for patients with HAE-C1INH-D in Latin America, every effort should be made to stimulate research. This year has seen the second edition of the Latin American Meeting on Hereditary Angioedema and the launch of the Latin American Hereditary Angioedema Association.

These initiatives are expected to improve the prognosis and quality of life of patients with HAE-C1INH-D in Latin America.

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Previous Presentations

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References

1. Bowen T, Cicardi M, Farkas H, Bork K, Longhurst HJ, Zuraw B, Aygoeren-Pürsün E, Craig T, Binkley K, Hebert J, Ritchie B, Bouillet L, Betschel S, Cogar D, Dean J, Devaraj R, Hamed A, Kamra P, Keith PK, Lacuesta G, Leith E, Lyons H, Mace S, Mako B, Neurath D, Poon MC, Rivard GE, Schellenberg R, Rowan D, Rowe A, Stark D, Sur S, Tsai E, Warrington R, Wasserman S, Ameratunga R, Bernstein J, Björkander J, Brosz K, Brosz J, Bygum A, Caballero T, Frank M, Fust G, Harmat G, Kanani A, Kreuz W, Levi M, Li H, Martinez-Saguer I, Moldovan D, Nagy I, Nielsen EW, Nordenfelt P, Reshef A, Rusicke E, Smith-Foltz S, Späth P, Varga L, Xiang ZY. 2010 International consensus algorithm for the diagnosis, therapy and management of hereditary angioedema. *Allergy Asthma Clin Immunol*. 2010 Jul 28;6(1):24-36.
2. Grumach AS, Valle SO, Toledo E, de Moraes Vasconcelos D, Villela MM, Mansour E, Pinto JA, Campos RA, França AT; On behalf of group interested on HAE (GINHA). Hereditary angioedema: first report of the Brazilian registry and challenges. *J Eur Acad Dermatol Venereol*. 2013;27(3):e338-44.
3. Fabiani JE, Avigliano A, Dupont JC. Hereditary Angioedema. Long Term follow-up of 88 patients. Experience of the Argentine Allergy and Immunology Institute Allergol Immunopathol (Madr). 2000;28(5):267-71.
4. Majluf-Cruz A, Nieto-Martínez S. Long-term follow up analysis of nadropin for hereditary angioedema. A preliminary report. *Intern Immunopharmacol*. 2011;11:1127-32.
5. Pappalardo E, Caccia S, Suffritti C, Tordai A, Zingale LC, Cicardi M. Mutation screening of C1 inhibitor gene in 108 unrelated families with hereditary angioedema: functional and structural correlates. *Mol Immunol*. 2008 Aug;45(13):3536-44.
6. López-Lera A, Garrido S, Roche O, López-Trascasa M. SERPING1 mutations in 59 families with hereditary angioedema. *Mol Immunol*. 2011 Oct;49(1-2):18-27.
7. Agostoni A, Cicardi M. Hereditary and acquired C1-inhibitor deficiency: biological and clinical characteristics in 235 patients. *Medicine (Baltimore)*. 1992 Jul;71(4):206-15.
8. Cugno M, Zanichelli A, Bellatorre AG, Griffi ni S, Cicardi M. Plasma biomarkers of acute attacks in patients with angioedema due to C1-inhibitor deficiency. *Allergy*. 2009;64(2):254-7.
9. Cicardi M, Craig TJ, Martinez-Saguer I, Hébert J, Longhurst HJ. Review of recent guidelines and consensus statements on hereditary angioedema therapy with focus on self-administration. *Int Arch Allergy Immunol*. 2013;161 Suppl 1:3-9.
10. Craig T, Aygören-Pürsün E, Bork K, Bowen T, Boysen H, Farkas H, Grumach A, Katelaris CH, Lockey R, Longhurst H, Lumry W, Magerl M, Martinez-Saguer I, Ritchie B, Nast A, Pawankar R, Zuraw B, Maurer M. WAO Guideline for the Management of Hereditary Angioedema. *World Allergy Organ J*. 2012 Dec;5(12):182-99.

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Anaphylaxis Mediated by Thaumatin-like Proteins

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Key words: Anaphylaxis. Thaumatin.

Palabras clave: Anafilaxia. Taumatinas.

Thaumatin-like proteins (TLPs) are found in many plant foods. They belong to pathogenesis-related (PR) group 5 and have a molecular weight of 20-30 kDa and a very compact 3-dimensional structure held together by 8 disulfide bridges. Their high stability during protein digestion, heat treatment, and exposure to acidic pH values enables them to remain in food after cooking or industrial processing. TLPs have been linked to various food allergies [1,2].

We describe a case of severe allergy to taxonomically unrelated plant foods in which evidence points to TLP as a possible causative panallergen. A 31-year-old woman who worked at her family's bakery was diagnosed 12 years ago with rhinitis and occupational asthma due to wheat flour allergy. Since then, her duties have been limited to bread distribution, and she has remained virtually asymptomatic.

Eight years ago, immediately after eating a banana, she experienced chest tightness and facial edema, which resolved a few hours after treatment in the emergency department. Six years ago, 10 minutes after eating grapes, she presented chest tightness, itchy throat and ears, vomiting, and abdominal pain. Red wine provoked a similar episode. She

also experienced a reaction to pear, in which she developed chest tightness, facial edema, abdominal pain, and dyspnea. On several occasions, she experienced intense abdominal pain a few minutes after eating tomato, apple, garlic, onion, carrot, chestnut, and lettuce. Peanuts, hazelnuts, almonds, and walnuts caused oral allergy syndrome, although no cofactors were identified. She tolerates peach and kiwi, is currently avoiding all the above-mentioned foods, and has remained asymptomatic.

Prick-prick test results were positive for banana, grape, pear, garlic, tomato, chestnut, peach, and kiwi. The results of a CAP assay (ThermoFisher) revealed values of 11 kU_A/L to banana, 17 kU_A/L to grape, 36 kU_A/L to pear, 5 kU_A/L to tomato, and 4 kU_A/L to peanut and hazelnut. The results of an ISAC CR-102 assay (ThermoFisher) showed 4.4 ISU to kiwi thaumatin (Act d 2). Specific IgE to panallergens in vivo and in vitro was ruled out for profilin, lipid transfer proteins (LTP), and Bet v 1 homologues.

Immunodetection with a serum sample showed IgE-binding bands of 20-40 kDa in kiwi, banana, chestnut, peach, apple, peanut, and birch and mugwort pollen. Similarly sized bands were recognized by TLP-specific antibodies (Figure).

Given that sensitization to multiple plant foods was suspected of being caused by TLPs, the patient's serum (1:2 dilution) was evaluated using an in-house allergen microarray including a battery of TLPs [3]. The tests revealed specific IgE to Act d 2 (505 fluorescence units [FU]), Mus a 4 (751 FU), chestnut TLP (2.238 FU), and *Platanus* pollen TLP (552 FU). A negative value was obtained with Cup a 3, Mal d 2, and hazelnut TLP.

Sensitization to TLPs was confirmed by skin prick test with purified thaumatin (50 µg/mL) [3], which showed a positive result to Mus a 4 (10×5 mm wheal) and Act d 2 (13×8 mm), although no response was observed to Pru p 2.02 (peach TLP) or chestnut TLP.

Several factors point to TLPs as the principal cause of the allergic manifestation in the case we report. First, the presence of TLPs in the foods involved is indicative of their role; indeed, TLPs have been described in apple [4], cherry [5], kiwi [6],

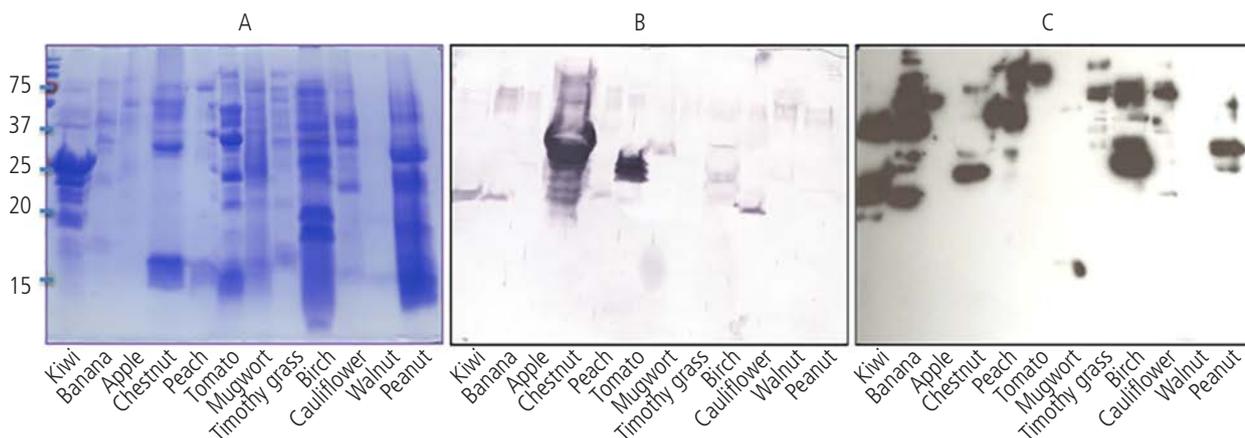


Figure. Protein extracts (phosphate-buffered saline, 10 mM; pH 7.4; 30 µg) from kiwi, banana, chestnut, apple, peach, tomato, mugwort, timothy grass, birch pollen, cauliflower, walnut, and peanut were separated using SDS-PAGE (15%) and stained with Coomassie brilliant blue (A). Replicas were electrotransferred and incubated antibodies produced against chestnut TLP (dilution 1:10 000 [B]) and the patient's serum (dilution 1:3 [C]).

banana [7], chestnut, peach [8], lettuce, hazelnut, wheat, grape, olive, and pepper, most of which led to symptoms in the present case. Second, the results of skin tests to the foods involved and purified TLPs were positive. Third, we identified specific IgE to the TLPs of various foods in immunoblotting. Finally, the microarray assay revealed specific IgE to the TLP of banana, kiwi, and chestnut. Given the finding of allergy to several taxonomically unrelated plant foods in a patient sensitized to thaumatin in the absence of sensitization to other panallergens (LTP, profilin, and Bet v 1 homologues), we can conclude that thaumatin was the primary panallergen responsible for these allergic reactions.

TLP has also been described in the pollen of cypress, birch, mugwort, olive, and plane [9]. Our patient had no clinical allergy to pollen, although she did have positive skin test results to birch, mugwort, and olive. In a recent study, Palacin et al [3] found that in some areas of Spain, up to 50% of fruit-allergic patients—especially those who are allergic to peach—had elevated levels of specific IgE to Pru p 2.02. The analysis of the sensitization profile of TLP revealed that fruit-allergic patients showed a strong positive response to several TLPs, although this response tended to be more frequent in patients with pollinosis.

It is striking that the patient had previously experienced occupational asthma due to allergy to wheat flour. TLP has been described as an allergen linked to baker's asthma [10].

To our knowledge, this is the first case report in which allergy to TLP has emerged as a putative panallergen responsible for a severe allergic reaction.

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References

- Breiteneder H. Thaumatin-like proteins -- a new family of pollen and fruit allergens. *Allergy*. 2004;59:479-81.
- Perri F, Romitelli F, Rufini F, Secundo F, Di Stasio E, Giardina B, Vitali A. Different structural behaviors evidenced in thaumatin-like proteins: a spectroscopic study. *Protein J*. 2008;27:13-20.
- Palacín A, Rivas LA, Gómez-Casado C, Aguirre J, Tordesillas L, Bartra J, Blanco C, Carrillo T, Cuesta-Herranz J, Bonny JA, Flores E, García-Alvarez-Eire MG, García-Nuñez I, Fernández FJ, Gamboa P, Muñoz R, Sánchez-Monge R, Torres M, Losada SV, Villalba M, Vega F, Parro V, Blanca M, Salcedo G, Díaz-Perales A. The involvement of thaumatin-like proteins in plant food cross-reactivity: A multicenter study using a specific protein microarray. *PLoS One*. 2012;7(9):e44088.doi:10.1371/journal.pone.0044088.
- Oberhuber C, Ma Y, Marsh J, Rigby N, Smole U, Radauer C, Alessandri S, Briza P, Zuidmeer L, Maderegger B, Himly M, Sancho AI, van Ree R, Knulst A, Ebner C, Shewry P, Mills EN, Wellner K, Breiteneder H, Hoffmann-Sommergruber K, Büblin M. Purification and characterization of relevant natural and recombinant apple allergens. *Mol Nutr Food Res*. 2008;52(Suppl 2):S208-19.
- Inschlag C, Hoffmann-Sommergruber K, O'Riordain G, Ahorn H, Ebner C, Scheiner O, Breiteneder H. Biochemical characterization of Pru a 2, a 23-kD thaumatin-like protein representing a potential major allergen in cherry (*Prunus avium*). *Int Arch Allergy Immunol*. 1998;116:22-8.
- Gavrovic-Jankulovic M, Cirkovic T, Vuckovic O, Atanasković-Marković M, Petersen A, Gojgić G, Burazer L, Jankov RM. Isolation and biochemical characterization of a thaumatin-like kiwi allergen. *J Allergy Clin Immunol*. 2002;110: 805-10.
- Palacín A, Quirce S, Sánchez-Monge R, Bobolea I, Díaz-Perales A, Martín-Muñoz F, Pascual C, Salcedo G. Sensitization profiles to purified plant food allergens among pediatric patients with allergy to banana. *Pediatr Allergy Immunol*. 2011;22:186-95.
- Palacín A, Tordesillas L, Gamboa P, Sanchez-Monge R, Cuesta-Herranz J, Sanz ML, Barber D, Salcedo G, Díaz-Perales A. Characterization of peach thaumatin-like proteins and their identification as major peach allergens. *Clin Exp Allergy*. 2010;40:1422-30.
- Cortegano I, Civantos E, Aceituno E, Del Moral A, López E, Lombardero M, Del Pozo V, Lahoz C. Cloning and expression of a major allergen from *Cupressus arizonica* pollen, Cup a 3, a PR-5 protein expressed under polluted environment. *Allergy*. 2004;59:485-90.
- Salcedo G, Quirce S, Díaz-Perales A. Wheat allergens associated with Baker's asthma. *J Investig Allergol Clin Immunol*. 2011;21:81-92.

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Allergy to Galactose-Alpha-1,3-Galactose: Clinical Features and the Diagnostic Value of Cetuximab

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Key words: Galactose-alpha-1.3-galactose. Basophil activation test. Cetuximab. Meat allergy. Tick bite.

Palabras clave: Galactosa-alfa-1.3-galactosa. Test de activación de basófilos. Cetuximab. Alergia a carne. Picadura de garrapata.

The diagnosis of mammalian meat allergy due to galactose-alpha-1,3-galactose (alpha-gal) [1] is difficult because skin tests with commercial meat extracts have low sensitivity. The aim of the present study was to investigate the usefulness of cetuximab (anti-EGFR) [2], a drug rich in alpha-gal, by means of the performance of skin tests and the basophil activation test (BAT) with this drug.

We studied 10 consecutive cases in white adults who developed delayed allergic reactions after eating mammalian meat (4-6 hours) and yoghurt (2-3 hours) (Table 1). Predominantly, the patients were middle-aged men, living or working in the countryside. They had all been bitten by ticks and 3 reported that they seemed to be particularly prone to tick bites.

Compounding factors were present in 8 of the 10 patients (Table 1): exercise in 3 cases and intake of nonsteroidal anti-inflammatory drugs (NSAIDs) in 5. With regard to dairy products that also seem to contain alpha gal [3], 2 patients developed allergy 2 hours after yoghurt ingestion during the study.

No systemic reactions were observed to tick bites. Three patients developed long-standing cutaneous reactions with episodic reactivations.

The patients underwent skin prick tests to total cow milk (IPI, Spain, 10 mg/mL), β -lactoglobulin and alpha lactalbumin (IPI, 5 mg/mL), casein (IPI, 10 mg/mL), bovine serum albumin (Sigma Aldrich Spain, 10 mg/mL) and meat, namely, meat mix and beef (Bial, Spain, 20 mg/mL), rabbit (Leti, Spain, 10 mg/mL), chicken (Leti, 20 mg/mL), pork serum (Leti, 50%), and cooked lamb meat. All patients showed doubtful or negative skin prick tests to the meat extracts; bovine serum albumin, and milk fractions were also negative. Prick tests with total cow milk were positive in 5 patients.

Skin prick tests were positive in all cases at 5 mg/mL [4] with cetuximab (Erbitux, Merck S.L.). An intradermal test (5 μ g/mL) [4] was performed in patients with shorter papule diameters (3-4 mm) and all the results were positive. Negative controls were carried out, with no reactions.

Six of the patients had blood type O+, 2 had type O-, and 2 had type A+.

Specific IgE to cow milk, bovine serum albumin, bromelain, and meat (lamb, pork, beef, rabbit, and chicken)

was measured by the CAP system (Phadia-Thermo Fisher). Alpha-gal was also measured by a CAP system, generously provided by Phadia-Thermo Fisher. Specific IgE to mammalian meats was positive in 9 of the 10 patients (range, 0.70-17 kU_A/L), and total milk was positive in 8 of the 10 patients. Specific IgE to alpha-gal was positive in all cases (range, 1.27 kU_A/L- >100 kU_A/L) and negative to bromelain, bovine serum albumin, and chicken meat.

BAT results were assessed by analyzing CD63 expression by basophils after in vitro allergen-specific stimulation with different cetuximab concentrations spanning several log scales, mimicking in vivo concentrations (0.25, 0.125 and 0.025 mg/mL) [2]. The technique was performed without bovine serum albumin. A monoclonal anti-IgE antibody (Sigma-Aldrich) at 1 mg/mL and N-formyl-methionyl-leucyl-phenylalanine (fMLP) (Sigma-Aldrich) at 1 mmol/L, were used as positive controls. Samples were acquired on a BD FACS flow cytometer (BD Biosciences), and at least 500 CD123^{high}HLA-DR^{dim} basophils per sample were analyzed. Activated basophils were identified additionally as CD63⁺. The test was carried out in 6 healthy individuals to exclude the ability of cetuximab to elicit nonspecific activation of basophils or the presence of nonspecific stimulatory components in the cetuximab presentation.

BATs with cetuximab were positive (stimulation range, 39%-93.7%) in all evaluable cases (6/10). Nevertheless, the patient with sIgE class 0 to beef and 0.14 kU_A/L to pork presented a positive BAT (89 %) with cetuximab (Table 1). A negative response to cetuximab by BAT was observed in 6 healthy controls. Surprisingly, 4 out of 10 patients showed no response to the positive control with anti-IgE in BAT and were therefore not evaluable.

IgE to alpha-gal is a good specific marker in the diagnosis of this kind of mammalian meat allergy, but unfortunately at the time the study was designed, the technique was not commercially available. The highest level of IgE to meat was against beef. However, this result is not specific because it does not discriminate between the possible allergens (alpha gal, serum albumins, or gammaglobulins).

Our results are consistent with those of previous studies confirming the usefulness of skin tests to cetuximab [4,6], and in addition we provide new effective results using BAT with cetuximab. BAT results were higher than those observed previously with mammalian meats extracts [5]. In our study, BATs to cetuximab were positive in 100% of the patients that could be evaluated (6/10), and the stimulation percentage was considerable (39%-93.7%).

Clinical symptoms were delayed, which is consistent with previously reported cases of patients with IgE antibodies to alpha-gal [6] and with reports of *Anisakis simplex* allergy [7]. In both series, most of the patients were nonatopic adults, and compounding factors (NSAIDs and exercise) were involved in the triggering of reactions.

Previous studies have emphasized the role of tick bites in the pathogenesis of mammalian meat allergy [6]. In Spain, only 1 small series involving 5 patients, mostly bitten by ticks, has been described [8], but the environmental conditions in the area described are similar to those in ours, possibly explaining the new cases observed in the north of Spain.

Table. Sex, Age, Blood Group, Clinical Data and In Vivo and In Vitro Tests Results

P	Age, y Sex BG	Clinical Features	Implicated Food and Cofactors	Milk/Meat Skin Test Results	Cetuximab Skin Tests Results	Inmunocap (kU _A /L, Class)	BAT (%)
1	27 ♀ 0+	Urticaria	Beef + exercise	Milk: 3x3 mm Raw and cooked meats: negative	Prick: 4x3 mm ID: 8x8 mm	IgE alpha-gal: 51.2 (5) Class 3: beef, pork, and lamb Class 2: rabbit and milk Class 0: BSA	73.1
2	32 ♂ 0+	Urticaria and inguinal pruritus	Pork and lamb + NSAID	Milk: negative Meat mix : 3x3 mm	Prick: 4x4 mm ID: 6x7 mm	IgE alpha-gal: 24.5 (4) Class 4: beef Class 3: pork and lamb Class 2: milk Class 0: BSA	NE
3	34 ♂ 0-	Urticaria	Pork kidneys Pork + exercise	Milk, meat, and BSA: negative	Prick: 5x5 mm ID: 11x9 mm	IgE alpha-gal: 7.00 (3) Class 2: beef, pork and lamb Class 1: milk Class 0: poultry, BSA	39
4	35 ♂ 0+	Urticaria, pruritus, and dyspnea	Lamb + exercise	Milk: 3x3 mm Meat mix, BSA: negative	Prick: 4x3 mm ID: 9x8 mm	IgE alpha-gal: 100 (6) Class 3: beef, pork, lamb, and milk Class 0: BSA	NE
5	39 ♂ 0+	Urticaria and severe abdominal pain	Ham + NSAID Pork sausage Beef, rabbit Yoghurt	Milk: 3x3 Pork: 6x5 Other meats: negative	Prick: 7x5 mm ID: not done	IgE alpha-gal: 25.2 (4) Class 2: beef, pork, lamb, rabbit and milk Class 0: bromelain, BSA	63
6	42 ♂ A+	Urticaria, redness, pruritus stomach pain, diarrhea, and vomiting	Pork kidneys Hamburger Beef + NSAID	Milk: negative Beef: 5x5 Pork: 6x5 Rabbit: 3x3	Prick: 5x4 mm ID: 9x8mm	IgE alpha-gal: 24.0 (4) Class 3: beef, pork Class 2: rabbit, lamb, and milk Class 1: cat dander Class 0: BSA	NE
7	48 ♂ A+	Palmoplantar pruritus and urticaria	Pork kidneys Yoghurt + ibuprofen	Milk: 3x3 Meat mix: 3x3	Prick: 7x7 mm ID: 15x7 mm	IgE alpha-gal: 1.27 (2) Beef: 0.19 kU _A /L Pork: 0.14 kU _A /L Class 0: milk and BSA	89
8	56 ♂ 0-	Genital and limb angioedema	Roast lamb and cow tripe	Milk: negative Meat mix: negative	Prick: 6x5 mm ID: not done	IgE alpha-gal: 54.5 (5) Class 5: beef and cat dander Class 4: pork Class 2: milk Class 0: BSA	74
9	60 ♂ 0+	Urticaria	Lamb + ibuprofen	Milk: 5x4 Meat mix : 6x4 Pork: 3x2	Prick: 3x3 mm ID: 10x7 mm	IgE alpha-gal: 1.37 (2) Class 2: beef Class 1: pork Class 0: milk, BSA, and bromelin	93.7
10	61 ♀ 0+	Urticaria, angioedema, pruritus, and vomiting	Pork and rabbit	Milk: negative Meat mix: negative	Prick: 4x5 mm ID: 6x6 mm	IgE alpha-gal: 34.2 (4) Class 3: beef, cat dander Class 2: pork Class 1: milk Class 0: rabbit	NE

Abbreviations: Alpha-gal, galactose-alpha-1,3-galactose; BAT, basophil activation test; BG, blood group; BSA, bovine serum albumin; ID, intradermal; NE, not evaluable; NSAID, nonsteroidal anti-inflammatory drug; P, patient number.

Alpha-gal has a similar structure to the B blood group antigen [9], but remarkably there were no patients with this blood group in our series. Eighty percent of the patients had blood type O, while the remaining 20 % had blood type A.

We did not perform an oral challenge due the positivity of the tests and the numerous episodes reported, in which alpha-gal was the only allergen involved.

In conclusion, based on our experience, BAT and skin tests with cetuximab help in the diagnosis of hypersensitivity

to alpha-gal, in accordance with alpha-gal IgE results. Considering the lack of alpha-gal prick testing, a positive skin test result with cetuximab is currently a good approach to diagnosis. Additionally, BAT to cetuximab is more sensitive and specific than BAT to mammalian meat. With regard to future treatment, our recommendation would be for these patients to avoid not only mammalian meats, but also dairy products in the event of allergic reactions to these products, as well as drugs that contain alpha-gal or any recombinant molecule expressed in a heterologous cell line that can constitutively present alpha-gal [10].

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Chung CH, Mirakhor B, Chan E, Le QT, Berlin J, Morse M, Murphy BA, Satinover SM, Hosen J, Mauro D, Slebos RJ, Zhou Q, Gold D, Hatley T, Hicklin DJ, Platts-Mills TA. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. *N Engl J Med*. 2008;358:1109-17.
2. Tan AR, Moore DF, Hidalgo M, Doroshow JH, Poplin EA, Goodin S, Mauro D, Rubin EH. Pharmacokinetics of cetuximab after administration of escalating single dosing and weekly fixed dosing in patients with solid tumors. *Clin Cancer Res*. 2006;12: 6517-22.
3. Kennedy JL, Stallings AP, Platts-Mills TA, Oliveira WM, Workman L, James HR, Tripathi A, Lane CJ, Matos L, Heymann PW, Commins SP. Galactose- α -1,3-galactose and delayed anaphylaxis, angioedema, and urticarial in children. *Pediatrics*. 2013; 131:e1545-52.
4. Jacquenet S, Moneret-Vautrin DA, Bihain BE. Mammalian meat-induced anaphylaxis: clinical relevance of anti-galactose-alpha-1,3-galactose IgE confirmed by means of skin tests to cetuximab. *J Allergy Clin Immunol*. 2009;124:603-5.
5. Kim JH, An S, Kim JE, Choi GS, Ye YM, Park HS. Beef-induced anaphylaxis confirmed by the basophil activation test. *Allergy Asthma Immunol Res*. 2010;2:206-8.
6. Commins SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD, Woodfolk JA, Platts-Mills TA. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose-alpha-1,3-galactose. *J Allergy Clin Immunol*. 2009;123:426-33.
7. Audicana MT, Kennedy MW. Anisakis simplex: from obscure infectious worm to inducer of immune hypersensitivity. *Clin Microbiol Rev*. 2008;21:360-79.
8. Nunez R, Carballada F, Gonzalez-Quintela A, Gómez-Rial J, Boquete M and Vidal C. Delayed mammalian meat-induced anaphylaxis due to galactose- α -1,3-galactose in 5 European patients. *J Allergy Clin Immunol*. 2011;128:1122-24.
9. Milland J, Sandrin MS. ABO blood group and related antigens, natural antibodies and transplantation. *Tissue Antigens*. 2006;68:459-66.
10. Jefferis, R. Glycosylation as a strategy to improve antibody-based therapeutics. *Nat Rev Drug Discov*. 2009;8:226-34.

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Anaphylaxis Due to *Eruca sativa*

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Key words: Food allergy. Anaphylaxis. Rocket. Cross-reactivity.

Palabras clave: Alergia alimentaria. Anafilaxia. Rúcula. Reactividad cruzada.

Arugula (rocket) allergy is a rare entity. Four types of arugula have been described, namely, *Diplotaxis tenuifolia*, *Diplotaxis muralis*, *Diplotaxis erucooides*, and *Eruca sativa*. *E sativa* belongs to the Brassicaceae (Cruciferae) family, which includes other common foods, such as cauliflower, cabbage, broccoli, turnip, and mustard.

We present the case of a 35-year-old woman, an office worker, with a history of allergic asthma and rhinitis in May and June with sensitization to olive pollen and house dust mites. The patient presented generalized urticaria, angioedema, and bronchospasm requiring treatment in the emergency room immediately after ingestion of arugula and goat cheese salad. No further information about the treatment or clinical situation of the patient was available.

A year earlier, the patient had experienced facial urticaria and pruritus after eating meat and a salad (lettuce, tomato and onion); the symptoms resolved without treatment in a couple of hours. The patient experiences oral allergy syndrome with tree nuts and raw cabbage. She tolerates other Brassicaceae (mustard, broccoli and cauliflower), beans, peas, fruits, and other types of lettuce.

We performed skin prick tests (SPTs) with commercial extracts (Leti Laboratories) to hazelnut, chestnut, peanut, soy, lentil, mustard, and peach, and prick to prick tests with raw arugula (*E sativa*), onion, cabbage, cauliflower, broccoli, and turnip.

Given the occurrence of anaphylaxis, we also measured, with the patient's informed consent, total serum tryptase, total serum IgE, and serum specific IgE to peach, peach lipid transfer protein, peanut, chestnut, hazelnut, soy, shrimp, cabbage, cauliflower, and broccoli, with profiling done by either the CAP or microarray (ISAC) technique (Thermo Fisher Scientific).

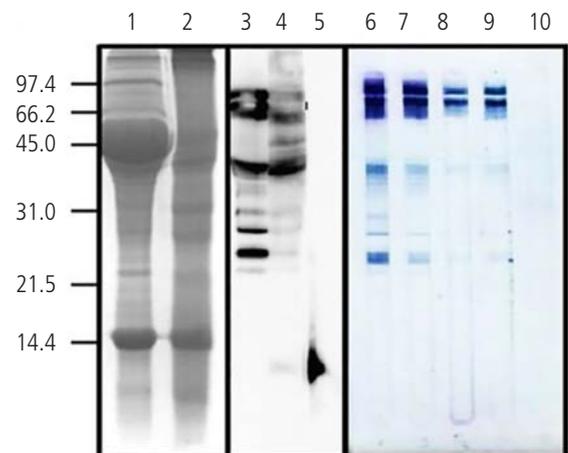
An extract with *E sativa* leaves was prepared following internal procedures (Leti Laboratories), and characterized by SDS-PAGE (Figure) under reducing and nonreducing conditions. A serum sample from the patient was incubated with the extract and the allergenic profile analyzed (Figure). Cross-reactivity studies were also performed with other members of the *Brassicaceae* family. Arugula extract was inhibited with 40 µg of cabbage, cauliflower, and broccoli (Figure).

As the patient had a history of asthma and presented with bronchospasm, we performed a baseline spirometry test and measured fraction of exhaled nitric oxide (Fe_{NO}) by Aerocrine NIOX MINO. The lung function results in the baseline

spirometry were a forced vital capacity (FVC) of 2.63 L (74%), forced expiratory volume in the first second (FEV₁) of 1.91 L (62%), and a FEV₁/FVC ratio of 72.5%. A postbronchodilator test was positive, with an increase of 510 mL (12%) in FEV₁. Fe_{NO} was 22 ppb (normal, <30 ppb).

The STP and prick to prick test results were positive (wheal >3 mm) to hazelnuts, chestnut, peanut, soy, lentil, mustard, peach, arugula (*E sativa*), raw onion, raw cabbage, raw cauliflower, raw broccoli, and raw turnip. Specific IgE by CAP was positive (>0.35 kU/L) to rPru p 3 (7.66 kU/L), *Platanus acerifolia* (13.6 kU/L), *Olea europea* (0.35 kU/L), mustard (5.12 kU/L), arugula (0.29 kU/L), broccoli (1.28 kU/L), cauliflower (2.32 kU/L), and cabbage (3.17 kU/L). Specific IgE by microarray was positive (>0.3 ISU) to nOle e 1 (0.9 ISU), nPla a 2 (1.2 ISU), nDer p 2 (8.1 ISU), rDer f 2 (6.2 ISU), rEur m 2 (0-5 ISU), nApi m 1 (1.7 ISU), nPru p 3 (3.5 ISU), rCor a 8 (1.2 ISU), and Art v 3 (0.6 ISU). Total serum tryptase was 10.3 IU/L and total serum IgE was 380 kU/L.

The arugula extract contained 346 µg of protein/mg of freeze-dried material. The SDS-PAGE revealed several bands, with the most prominent at 15 and 45 kDa. It also revealed bands at approximately 25, 40, and 66 kDa.



SDS-PAGE (15%T) Coomassie G-250

1. Rocket reducing conditions, 40 µg of protein.
2. Rocket, nonreducing conditions, 40 µg of protein.

Western blot (SDS 15%T) with patient's serum, dilution 2/3 solid phase:

3. Rocket reducing conditions, 40 µg of protein.
4. Rocket nonreducing conditions, 40 µg of protein.
5. Peach lipid transfer protein, 20 µg.

Inhibition Western blot (SDS 15%T):

6. Rocket solid phase (40 µg of protein) incubated with serum 2/3.
7. Rocket solid phase (40 µg of protein) incubated with serum 2/3 inhibited with cabbage (40 µg of protein).
8. Rocket solid phase (40 µg of protein) incubated with serum 2/3 inhibited with cauliflower (40 µg of protein).
9. Rocket solid phase (40 µg of protein) incubated with serum 2/3 inhibited with broccoli (40 µg of protein).
10. Negative control. PBS-Tween 0.1%.

Figure. SDS-PAGE results.

The allergenic profile of the individual showed several bands coinciding with the most representative allergens in the SDS-PAGE. Inhibition studies with cabbage, cauliflower, and broccoli showed partial inhibition of the main recognized bands, with the clearest inhibitor being broccoli.

This study shows the presence of specific IgE to *E sativa* in our patient's serum that correlates with the allergic symptoms experienced after ingestion, which would explain the anaphylaxis. Although the levels of IgE to *E sativa* are low, we note the recognition of certain arugula allergens (21-97 kDa), as shown in the immunoblot (Figure). These allergens are partially inhibited by cauliflower, broccoli, and cabbage as shown in the inhibition immunoblot (Figure), suggesting a certain degree of cross-reactivity between these foods, although some allergens could be specific to *E sativa*. Further studies with a high number of serum samples are required.

We found few studies of arugula allergy in the literature, and most of these were case reports. In 1991, Pigatto et al [1] first described generalized urticaria in a patient who had prepared a salad containing arugula and had a positive SPT and radioallergosorbent test for *E sativa*. In 1998, Liccardi et al [2] described a case of intraoral and respiratory allergy after ingestion of *E sativa* in a patient sensitized to *Parietaria* species and Gramineae. They performed prick to prick tests, with positive results for *E sativa* and negative results for other foods in the same family (mustard, cabbage, and turnip). Finally, Brito et al [3] described 2 cases of allergic rhinoconjunctivitis and occupational asthma due to *D eruroides* in 2 vineyard workers.

We have presented the first case described in Spain of anaphylaxis due to *E sativa* in a patient sensitized to other Brassicaceae and with tolerance of mustard and cooked members of the Brassicaceae family, such as cauliflower and broccoli. We have shown the presence of specific IgE to *E sativa* and other Brassicaceae species and the presence of a certain degree of cross-reactivity between foods from the Brassicaceae family.

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Conflicts of Interest

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References

1. Pigatto PD, Bigardi A, Fumagalli M, Legori A, Altomare G. Ig-E mediated contact and generalized urticaria from *Eruca sativa*. *Contact Dermatitis*. 1991; 25: 191-2.
2. Liccardi G, Salzillo A, Mistrello G, Roncarolo D, D'Amato M, D'Amato G. Intraoral and respiratory allergy to *Eruca sativa* (Cruciferae family) in a subject with sensitization to pollen allergens. *J Allergy Clin Immunol*. 1998; 101: 559-60.
3. Brito FF, Mur P, Bartolomé B, Galindo PA, Gomez E, Borja J, Martinez A. Rhinoconjunctivitis and occupational asthma caused by *Diplotaxis eruroides* (wall rocket). *J Allergy Clin Immunol*. 2001; 108:125-7.

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ERRATUM:

Serum Specific IgE: A Biomarker of Response to Allergen Immunotherapy

G Ciprandi, M Silvestri

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The correct name of the second author is "Silvestri" not "Sivestri" as published.

Allergy to Rabbit Meat After Sensitization by Inhalation

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Key words: Food allergy. Meat allergy. Rabbit meat allergy. Sensitization by inhalation.

Palabras clave: Alergia alimentaria. Alergia a carne. Alergia a carne de conejo. Sensibilización por inhalación.

Allergy to mammalian meat is uncommon, although its prevalence in some European countries is increasing [1]. In most cases it begins in childhood and disappears within a few years [2].

We present the case of a 20-year-old woman who has experienced allergic rhinoconjunctivitis along with itching and skin erythema due to rabbit exposure since childhood. She also displays symptoms when exposed to dogs, cats, and horses. The allergic episodes are resolved with oral antihistaminic treatment and bronchial problems have never appeared. The patient experiences no symptoms in the absence of contact and keeps no animals in her home.

In April 2012, immediately after eating paella (containing rice, saffron, chicken, rabbit meat, tomatoes, green beans, artichoke, and butter beans [*Phaseolus lunatus*]), the patient developed urticaria, dyspnea, stridor, dysphonia, abdominal pain, and dizziness. Since then, she has not eaten rabbit meat, artichoke, green beans, or butter beans, and has tolerated the rest of the ingredients present in the paella (rice, saffron, chicken, and tomatoes).

Skin prick tests were carried out with aeroallergen extracts typically found in our region: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria alternata*, various pollens (*Platanus acerifolia*, *Olea europaea*, *Parietaria judaica*, *Cupressus arizonica*, *Salsola kali*, *Chenopodium album*, *Plantago lanceolata*, *Artemisia vulgaris*, and Gramineae pollens), and dog, cat, horse, and rabbit epithelium (Bial-Aristegui).

Skin tests were performed with extracts of rabbit meat, green beans, artichoke, and cow's milk, and the following purified proteins: peach lipid transfer protein (LTP), lactalbumin, lactoglobulin, and casein from cow (Bial-Aristegui). Prick by prick tests were also carried out with butter beans, green beans, and artichoke.

Levels of serum specific IgE (EAST technique, Bial-Aristegui) were determined to rabbit epithelium, urine, and meat; dog and cat epithelium; chicken, beef, and pork, as well as purified bovine serum albumin (BSA).

The molecular mass of the IgE-binding proteins was established using SDS-PAGE immunoblotting following the Laemmli method with meat extracts, rabbit epithelium and

urine, epithelium from other mammals (dog, cat, horse, and cow), and urine from cat and cow.

A cross-reactivity study was carried out with the immunoblotting-inhibition technique using rabbit meat extract in solid phase and rabbit epithelium and urine as the inhibitor phase.

Skin tests with aeroallergens were positive to Gramineae pollen and rabbit, dog, cat, and horse epithelium. Skin tests with foods were positive only to rabbit meat.

Serum specific IgE levels were only positive (>0.35 kU/L) to rabbit epithelium (38 kU/L), urine (13.6 kU/L), and meat (6 kU/L).

SDS-PAGE immunoblotting results revealed a 66-kDa IgE binding band (probably albumin) in rabbit meat, epithelium, and urine extracts, a 40-kDa IgE reactive band in rabbit meat and epithelium extracts, a 28-kDa band in meat and urine extracts, and a 14-kDa band in the urine extract only (Figure A). Low-intensity IgE reactive bands were detected in cat, horse, and cow epithelium extracts and in cow urine (results not shown).

Immunoblotting-inhibition results showed total IgE binding inhibition on rabbit meat extract when both rabbit epithelium and urine were used as inhibitors (Figure B). These results were presumably due to the presence of the same proteins in all of these extracts.

The paella ingredients to which no sensitization was detected (green beans, artichoke, and butter beans) were well tolerated after their reintroduction into the patient's diet.

A search of the medical literature revealed several cases related to animal meat allergy, some in patients with clinical or subclinical sensitization to animal epithelium [3,4]. Sensitization can occur through the digestive tract, inhalation, or dermal exposure. Most published cases have involved beef, and less frequently, other kinds of meat such as pork and horse meat. Allergy to meat from other mammals is extremely rare, and only isolated cases have been reported.

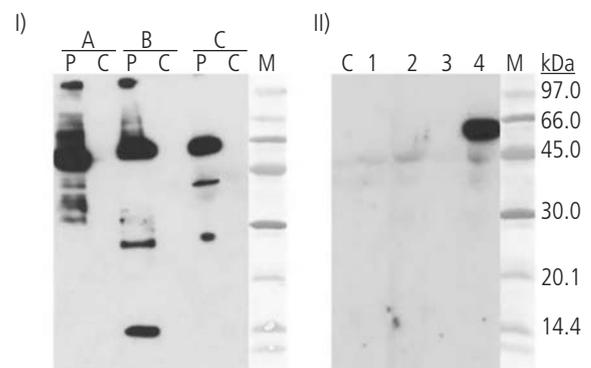


Figure 1. SDS-PAGE immunoblotting results. A, Rabbit epithelium extract. B, Rabbit urine extract. C, Rabbit meat extract. Lane P, Patient serum, Lane C, Control serum (pool of sera from nonatopic individuals). Lane M, Molecular mass marker. II, Immunoblotting-inhibition results. Rabbit meat extract in solid phase. Lane C, Control serum (pool of sera from nonatopic individuals). Lane 1, patient serum previously incubated with rabbit meat extract. Lane 2, Patient serum previously incubated with rabbit epithelium extract. Lane 3, Patient serum previously incubated with rabbit urine extract. Lane 4, Patient serum previously incubated with sunflower pollen extract. Lane M, Molecular mass marker.

In 2012, a case of facial angioedema after rabbit meat ingestion was described in a 49-year-old woman with no medical history of hypersensitivity to animal epithelium; the angioedema was assumed to be due to allergy to rabbit serum albumin [5].

Serum albumin is a panallergen which should be always taken into consideration in cases of allergy to mammalian meat and dander [6]. BSA, which has been defined as a thermolabile protein in most studies [3,4,7] accounts for most cross-reactivity between different kinds of mammalian meat and dander. Our patient tolerated the ingestion of other mammalian meats (beef, lamb, pork).

Other major allergens in addition to serum albumin have been described in rabbit, primarily in the animal's saliva, urine, and fur. These include Ory c 1 (a 17-18 kDa protein from the lipocalin group, which is one of the most important groups of airborne animal allergens), Ory c 2 (a 21-kDa protein, also a member of the lipocalin group) [8], and Ory c 3 (a 18-19 kDa lipophilin) [9].

Cases of mammalian meat allergy following sensitization to carbohydrates have been described in recent years. Cells from mammals other than primates contain the oligosaccharide galactose- α -1,3-galactose (α -gal). Sensitization to this epitope provokes anaphylaxis a few hours after the ingestion of red meat (beef, pork, lamb). In 2 cases, allergy to pork has been associated with rabbit meat [10].

In conclusion, we have presented a case of allergy to rabbit meat with previous sensitization to proteins from rabbit epithelium and urine by inhalation. The patient tolerates ingestion of beef and other mammalian meats. A positive skin test and serum specific IgE levels proved the existence of an IgE-mediated mechanism. SDS-PAGE immunoblotting revealed the presence of a 67-kDa IgE binding protein in rabbit meat, epithelium, and urine, which could correspond to rabbit serum albumin. The cross-reactivity results led us to assume that primary sensitization to rabbit epithelium and urine protein facilitated the subsequent allergic reaction to rabbit meat.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Previous Presentation

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References

- Restani P, Ballabio C, Tripodi S, Fiocchi A. Meat allergy. *Curr Opin Allergy Clin Immunol*. 2009;9:265-9.
- Fuentes Aparicio V, Sánchez Marcén I, Pérez Montero A, Baeza ML, de Barrio Fernández M. Allergy to mammal's meat in adult life: immunologic and follow-up study. *J Invest Allergol Clin Immunol*. 2005;15:228-31.
- Kanny G, de Hautecloque C, Moneret-Vautrin DA. Food anaphylaxis to bovine serum albumin. *J Allergy Clin Immunol*. 1998;101:137-9.
- Fiocchi A, Restani P, Riva E, Mirri GP, Santini I, Bernardo L, Galli CL. Heat treatment modifies the allergenicity of beef and bovine serum albumin. *Allergy*. 1998;53:798-802.
- Egüez MF, Cabeza NC, González MI, Botello MD, de Luque V, Guardia P. Paciente con angioedema facial tras ingesta de carne de conejo. *J Investig Allergol Clin Immunol*. 2012;22(suppl.1):141.
- Palacios Benito P, Álvarez Lovel MC, Martínez Cócera M, del Castillo Payá M, Romanillos RA. Allergy to meat. *Allergy*. 2002;57:858-9.
- Spitzauer S, Pandjaitan B, Söregi G, Mühl S, Ebner C, Dietrich K, Valenta R, Rumpold H. IgE cross-reactivities against albumins in patients allergic to animals. *J Allergy Clin Immunol*. 1995;96:951-9.
- Saleh H, Embry S, Nauli A, Atyia S, Krishnaswamy G. Anaphylactic reactions to oligosaccharides in red meat: a syndrome in evolution. *Clin Mol Allergy*. 2012;10:5. doi: 10.1186/1476-7961-10-5.
- Baker J, Berry A, Boscatto LM, Gordon S, Walsh BJ, Stuart MC. Identification of some rabbit allergens as lipocalins. *Clin Exp Allergy*. 2001; 31(2): 303-12.
- Hilger C, Kler S, Arumugam K, Revets D, Muller CP, Charpentier C, Lehnert C, Morisset M, Hentges F. Identification and isolation of a Fel d 1-like molecule as major rabbit allergen. *J Allergy Clin Immunol*. 2013 Jun 10. pii: S0091-6749(13)00681-7. doi: 10.1016/j.jaci.2013.04.034

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Anaphylaxis Due to Pentoxifylline

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Key words: Anaphylaxis. Pentoxifylline. Purines. Allergy.

Palabras clave: Anafilaxia. Pentoxifilina. Purinas. Alergia.

Pentoxifylline is a drug derived from purines that is used in the treatment of peripheral vascular disease and cerebrovascular disease [1]. There are also reports describing its use as adjunctive therapy in urticarial vasculitis due to its immunomodulatory effects [2].

We report the case of a 53-year-old man who was referred to our allergy unit following treatment with Pentoxifylline Belmac 400 mg for edema and pain in the right knee. Immediately after administration of the first dose, the patient experienced oropharyngeal itching, sneezing, and a foreign body sensation. After 15 to 20 minutes, he developed severe angioedema and erythema on the posterior trunk, head, and limbs, accompanied by respiratory distress with cough and dyspnea. He was treated with corticosteroids and antihistamines and the reaction gradually remitted until fully resolved. The patient did not recall having been treated with purines (mainly theophylline and allopurinol) in the past, and routinely tolerates, with no adverse effects, food and beverages containing caffeine and theobromine such as coffee, Coca-Cola, and chocolate.

Prick tests for a battery of foods, *Anisakis simplex*, and latex (ALK-Abelló SA) were negative. A prick test with pentoxifylline solution (20 mg/mL) was positive (Figure). The same test performed in 10 controls (5 atopic and 5 nonatopic) was negative in all cases. Prick and intradermal tests with aminophylline (19.32 and 0.19 mg/mL) and azathioprine (5 and 0.1 mg/mL) were negative. As we did not have allopurinol in solution, this drug was tested using the prick to prick technique and was negative. Oral tolerance tests with anhydrous theophylline and allopurinol up to a cumulative dose of 200 and 150 mg, respectively, showed adequate tolerance.

A basophil activation test was performed using a commercial kit (BASOTEST, Glycotope Biotechnology) according to the manufacturer's protocol. In brief, 100 μ L of the patient's heparinized whole blood was drawn into four 5-mL tubes and incubated in a water bath at 37°C for 10 minutes with 20 μ L of stimulation buffer. Afterwards, the samples were incubated with 100 μ L 1:200 and 1:100 dilutions of pentoxifylline, washing dilution as a negative control, and fMLP (N-formyl-Met-Leu-Phe) as a positive control for 20 minutes at 37°C. Then, degranulation was stopped by

incubating the samples on ice for 5 minutes, adding 20 μ L of staining reagent (anti CD63-FITC and anti-IgE-PE antibodies), and incubating the tubes for 20 minutes in an ice bath, covered to prevent exposure to light. Erythrocytes were lysed for 10 minutes before centrifugation (5 minutes at 250 g).

Flow cytometric analysis was performed at 488 nm on a FACScan flow cytometer (Becton Dickinson) and analyzed by CellQuest software. Data acquisition was performed on at least 500 basophils per sample and results were considered positive when the percentage of activated basophils was at least 5% and the stimulation index, calculated as the ratio between the percentage of activated basophils after stimulation and that of activated basophils at baseline, was 2 for at least 1 of the pentoxifylline concentrations used. The percentage of activated basophils at baseline was 1.28%, and the dilutions of pentoxifylline at 1:200 and 1:100 were 1.59% and 6.62%, respectively. The stimulation indices were 1.24 for IE1 (pentoxifylline at 1/200) and 5.17 for IE2 (pentoxifylline at 1/100).

A histamine release test (HRT) was performed for pentoxifylline according to the methods recommended by the manufacturer (ReFlaB/Biopharm) for research purposes. Briefly, the HRT was performed in a customized microtiter plate with glass fibers to provide a solid phase for histamine binding. The patient's heparinized whole blood was challenged by pentoxifylline at the highest concentration that did not induce nonspecific histamine release or interfere with the histamine analysis (0.15 mcg/mL). The released histamine was measured using HISTAREADER 501 (RefLab).

The possibility of a single- or double-blind specific challenge test with pentoxifylline was considered but ruled out because of ethical reasons, the existence of therapeutic alternatives, the fact that the drug is not essential therapy at present, and the patient's opinion.



Figure. Positive prick test with pentoxifylline solution (20 mg/mL).

There are isolated case reports of allergic reactions to methylxanthine derivatives [3-5]: One of these described a case of urticaria due to pentoxifylline in which diagnosis was established by intradermal and provocation tests [3]; the patient developed an itchy rash in the hour following the administration of the last cumulative dose (540 mg) of pentoxifylline. In a second case, the skin tests were negative and a single-blind, placebo-controlled oral challenge was performed [4]. The results were positive for pentoxifylline (cumulative dose, 600 mg) and negative for theophylline and allopurinol. In the present case, the provocation test was not performed due to the severity of the patient's symptoms. To our knowledge, there are no reports of IgE-mediated anaphylaxis due to pentoxifylline based on the correlation of clinical history and skin and basophil degranulation test results.

The appearance of signs and symptoms of anaphylaxis immediately after the administration of the first dose of pentoxifylline may possibly be explained by cross-reactivity with other purines. Although the patient did not recall having received these treatments, purines such as theophylline were widely used in Spain in the past. However, the results of the allergy tests conducted with chemically related drugs (theophylline, allopurinol, and azathioprine) do not support this hypothesis. It might be speculated that sensitization to pentoxifylline could be related to previous exposure to purines contained in different foodstuffs, such as caffeine [5-9]. In 1 report of urticaria caused by a cola drink and caffeine [5], no clear immune mechanism was identified. Although skin and challenge tests were positive, the authors did not rule out a nonallergic hypersensitivity mechanism. In another case of anaphylaxis due to caffeine in which the diagnosis was based on a prick test and the dot-blot technique, the results of both tests were positive for caffeine and cola and negative for cola without caffeine [6]. Daroca et al [10] reported an unusual reaction of fever induced by caffeine, with a positive oral challenge with caffeine and theophylline and a negative challenge with pentoxifylline. Based on the lack of typical clinical features and negative skin tests, the authors speculated about the possibility of a type III or IV rather than a type I reaction. The patient described in the current report tolerates foods and beverages containing caffeine.

In summary, pentoxifylline allergy should be considered in the assessment of patients with a similar profile to that described herein, especially when they are taking multiple drugs.

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Conflicts of Interest

Fernando Pineda is an employee of Diater Labs. The remaining authors declare that they have no conflicts of interest.

References

1. Ward A, Clissold SP. Pentoxifylline: A review of its Pharmacodynamic and Pharmacokinetic Properties, and its Therapeutic Efficacy. 1987. *Drugs* 34: 50-97.
2. Nürnberg W, Grabbe J, Czarnetzki BM. Urticarial Vasculitis Syndrome Effectively Treated with Dapsone and Pentoxifylline. 1995. *Acta Derm Venereol.* 75: 54-6.
3. Gonzalez-Mahave MD, Del Pozo A, Blasco T, Lobera M, Venturini. Urticaria due to Pentoxifylline. 2005. *Allergy.* 60:705.
4. Seoane-Lestón FJ, Añibarro-Bausela MB, Mugica-García MV, Aguliar-Martinez A. Urticaria caused by Pentoxifylline. 2009. *J Investigation Allergology Clinical Immunology.* Vol 19(1): 64-79.
5. Fernández- Nieto M, Sastre J, Quirce S. Urticaria caused by cola drink. 2002. *Allergy.* 57: 967-8
6. Infante S, Baeza ML, Calvo M, De Barrio M, Rubio M, Herrero T. Anaphylaxis due to caffeine. 2003. *Allergy.* 58: 681-2.
7. Caballero T, García-Ara C, Pascual C, Diaz-Pena JM, Ojeda A. Urticaria induced by caffeine. *J Investig Allergol Clin Immunol* 1993, vol 3 (3): 160-2.
8. Pola J, Subiza J, Armentia A, Zapata C, Hinojosa M, Losada E, Valdivieso R. Urticaria caused by caffeine. *Ann Allergy* 1988; 60 (3): 207-8.
9. Hinrichs R, Hunzelmann N, Ritzkowski A, Zollner TM, Krieg T, Scharffetter-Kochanek K. Caffeine hypersensitivity. *Allergy.* 2002 Sep;57(9):859-60.
10. Daroca P, Rodriguez J, Reaño M, Canto G, Miranda P. Fever from caffeine. *Allergy* 1996; 51: 189-191.

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Occupational Allergy to *Ephestia kuehniella* in the Biological Control Industry

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Key words: Biological control. *Ephestia kuehniella*. Feeding of predatory mites. Flour moth. Occupational asthma.

Palabras clave: Control biológico. *Ephestia kuehniella*. Alimentación de ácaros depredadores. Mariposa de la harina. Asma ocupacional.

Biological control of plagues has increased dramatically in our area as an alternative to the indiscriminate use of pesticides [1]. In recent years, the number of biofactories or commercial insectaries has grown steadily in our region: south-eastern Spain.

Biological control consists of plague repression using natural enemies, which can be predators (eg, mites), parasites, or pathogens. These enemies are raised and later introduced into greenhouses or crops to kill plagues. Eggs from *Ephestia kuehniella* (Mediterranean flour moth), an insect belonging to the Lepidoptera order (Figure A), are used to feed the predatory mites used in biological control. A patient visited our unit with rhinitis and asthma incurred while working in the biological control industry.

A 30-year-old white male entomologist who had been working in the laboratory of a biological control company for 3 years had started to experience sneezing, itching, watery nasal and ocular discharge, and nasal congestion 2 years earlier while handling *E kuehniella* eggs. Several months before his visit, he began to experience wheezing and dyspnea twice a week. He was not in contact with predatory mites, and his symptoms improved during holidays and weekends. Skin prick tests were performed with common aeroallergens, different types of flour (wheat, barley, rye, corn, and rice), and *E kuehniella* egg extract. These tests were positive to extracts from *Dermatophagoides pteronyssinus*, olive pollen, and *E kuehniella* egg extract. The patient had never experienced allergic symptoms when exposed to olive pollen or *D pteronyssinus*.

Protein extracts from *E kuehniella* egg were prepared by homogenization in phosphate buffer saline, dialyzation, and lyophilization. SDS-PAGE analysis was performed as described by Laemmli [2] and revealed protein bands ranging from 67 to 13 kDa.

Serum specific IgE levels were measured using the enzyme allergosorbent test against *E kuehniella* egg extract (HYTEC Specific IgE EIA kit, HYPOR Biomedical Ltd), yielding 74.2 kU/L.

SDS-PAGE immunoblotting was carried out with *E kuehniella* egg extract under reducing (with 2-mercaptoethanol) and nonreducing (without 2-mercaptoethanol) electrophoretic conditions. An IgE-binding band of 66 to 50 kDa and various IgE-reactive bands at 45 kDa, 14 kDa and 13 kDa were revealed (Figure B).

Baseline spirometry was normal and the bronchodilator test was negative. The patient had a baseline peak flow rate of approximately 670 mL. Peak flow rate was monitored for a month, and when the patient was in contact with *E kuehniella* eggs in the laboratory, he experienced asthma in the evenings and a reduction in peak flow rate of between 100 and 140 mL. Peak flow rate was also monitored for 2 weeks while the patient was on holidays and there were no changes in the baseline rate. Nonspecific bronchial hyperresponsiveness was not assessed.

Biological methods of fighting plagues using predatory mites are employed in 21 000 hectares of greenhouse and crops in our area, mainly due to increasing market demand for pesticide-free crops. Manipulation of *E kuehniella* eggs used to feed these predatory mites can cause sensitization among workers employed in this activity. We have demonstrated

A



B

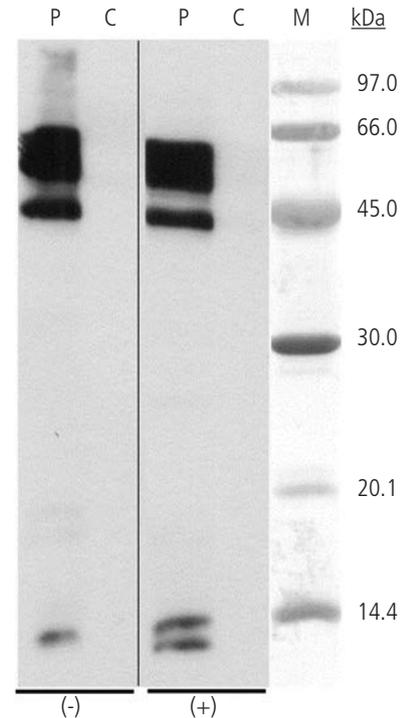


Figure. A, *Ephestia kuehniella* or Mediterranean flour moth. B, SDS-PAGE immunoblotting. Lane P, Patient serum; Lane C, Control serum (pool of sera from nonatopic subjects); Lane M, Molecular mass marker. (-) indicates samples without 2-mercaptoethanol; (+), samples with 2-mercaptoethanol.

the immunological IgE-mediated mechanism of our patient's pathology using a skin prick test, serum specific IgE levels, and immunoblotting results. We suggest that our patient was sensitized by inhalation exposure to *E kuehniella* eggs during handling at work. According to Lugo et al [1] this would be possible because *E kuehniella* egg proteins can be considered complete antigens and are able to evoke an IgE-mediated direct immune response. Furthermore, the dimension of egg particles allows them to be easily inhaled and they may therefore sensitize individuals through bronchial and nasal mucosal absorption [1].

Rhinitis and asthma usually have a latency period of 1 to 2 years, which supports the theory that sensitization occurs during occupational exposure [1], as probably happened with our patient.

After a 4-year follow-up study, Belisario et al [3] reported that the application of recommended preventive measures led to a decrease in skin test sensitization to *Ephestia* and *Orius* species, as well as a decrease in allergic diseases in a group of exposed workers employed in breeding insects for biological pest control. The authors also reported no allergy symptoms in recently employed workers who follow these measures. Our patient, however, became sensitized to *E kuehniella* even though he followed the recommended preventive measures (semi-closed cycles, gloves, protective masks, and goggles).

In the literature consulted we found the case of a baker sensitized to flour moth (*E kuehniella*) in which Mäkinen-Kiljunen et al [4] demonstrated an IgE-mediated mechanism by skin prick testing, specific IgE determination, and nasal challenge. Immunoblotting with the patient's serum revealed at least 7 intense IgE-binding bands with molecular masses of 86, 77, 65, 53, 43, 35, 22 kDa in whole flour moth extract. In our case, we demonstrated IgE-mediated sensitization by prick test and specific IgE to *E kuehniella* egg extract; the immunoblotting results showed IgE-binding bands of approximately 66-50, 45, 14, and 13 kDa. The lack of correspondence between the molecular masses of the IgE-binding bands detected in both studies might be explained by the difference in the allergenic sources used to prepare the extracts: *E kuehniella* bodies in the study by Mäkinen-Kiljunen et al and *E kuehniella* eggs in ours.

In 2004, Armentia et al [5] stated that sensitization to *Eurogaster* and *Ephestia* grain pests could be an important cause of asthma episodes experienced by certain patients (cereal stockers), and described the parasitized wheat as highly allergenic. Pala et al [6], in turn, identified *E kuehniella* as the responsible allergen in a polysensitized cereal stocker who experienced rhinitis and asthma following a specific inhalation challenge. Although we did not carry out a specific inhalation challenge, peak flow measurements showed a decreased rate when the patient was exposed to *E kuehniella*, which supports our diagnosis of occupational asthma due to *E kuehniella* egg sensitization.

We conclude that our patient is sensitized to *E kuehniella* eggs and have demonstrated an IgE-mediated mechanism by skin prick testing and the detection of serum specific IgE to *E kuehniella* egg extract.

Immunoblotting revealed IgE-binding bands when *E kuehniella* egg extract was incubated with the patient's sera. Furthermore, we detected a decrease in peak flow rate when the patient was in contact with *E kuehniella* eggs, so we think that he

developed occupational rhinitis and asthma due to *E kuehniella* egg sensitization. To the best of our knowledge, this is the first case of *E kuehniella* egg sensitization, with the detection of IgE-binding bands, to be reported in the biological control industry. More studies are necessary to determine if these bands are specific to *E kuehniella* eggs and responsible for producing allergic disease in workers at biological control facilities.

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Conflict of Interest

B Bartolomé works at the R&D Department of BIAL-Aristegui. The rest of authors declare that they have no conflicts of interest.

Previous Presentation

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References

1. Lugo G, Cipolla C, Bonfiglioli R, Sassi C, Maini S, Cancellieri MP, Raffi GB and Pisi E. A new risk of occupational disease: allergic asthma and rhinoconjunctivitis in persons working with beneficial arthropods. *Int Arch Environ Health*. 1994; 65: 291-94.
2. Laemmli UK: Cleavage of structural protein during assembly of the head of bacteriophage T4. *Nature* 1970 Aug 15; 227 (5259):680-5.
3. Belisario A, Cipolla C, Nucci C, Auletti G, Nobile M and Raffi GB. Follow-up di 4 anni in un gruppo di lavoratori con sensibilizzazione allergica addetti alla produzione di insetti per la lotta biologica. *Medicina del Lavoro*. 2001; 92, 2: 125-129.
4. Mäkinen-Kiljunen S, Mussalo-Rauhamaa H, Petman L, Rinne J and Haahtela T. A baker's occupational allergy to flour moth (*Ephestia kuehniella*). *Allergy*. 2001; 56: 696-700.
5. Armentia A, Lombardero M, Martínez C, et al. Occupational asthma due to grain pests *Eurygaster* and *Ephestia*. *J Asthma*. 2004. Feb; 41: 99-107.
6. Pala G, Pignatti P, Perfetti L, Caminati M, Moscato G. An uncommon case of occupational rhinitis and asthma. *EAAIC* 20010; DOI: 10.1594/eaacinet2010/CR/1-070410.

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Naproxen Increases the Severity of Food-Dependent Exercise-Induced Anaphylaxis: A Case Report

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Key words: Food allergy. Naproxen. Aspirin. Food-Dependent Exercise-Induced Anaphylaxis (FDEIA).

Palabras clave: Alergias alimentarias. Naproxeno. Aspirina. Anafilaxia inducida por el ejercicio dependiente de alimentos.

Food-dependent exercise-induced anaphylaxis (FDEIA) is a rare syndrome that was first reported in 1979 by Maultiz et al [1], who presented the case of a patient in whom anaphylactic symptoms were induced by exercise after ingestion of shellfish. In contrast, both ingestion of the causative food and strenuous exercise alone were well tolerated [1]. In 1983, Kidd et al [2] presented 4 patients with similar symptoms and introduced the term FDEIA into clinical practice. In some patients with FDEIA, the results of challenge with food and exercise are negative, whereas addition of aspirin or intake of aspirin without exercise triggers symptoms [3,4]. Thus, in FDEIA, aspirin can be considered an amplifying factor or even a substitute for exercise as a trigger of anaphylaxis. The mechanism by which this drug acts most probably depends on its pharmacological properties, namely, inhibition of cyclooxygenase-1, because reduced synthesis of prostaglandin E1 in the gastrointestinal

tract can result in increased absorption of food allergens [5,6]. Matsuo et al [7] recently demonstrated enhanced release of histamine from basophils by aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) in patients with FDEIA and urticaria compared with healthy controls and proposed a new mechanism for this phenomenon, namely, Syk kinase activation. To date, only aspirin has been reported as a cofactor of FDEIA, and the role of other NSAIDs has yet to be confirmed. We report a case of FDEIA in a patient in whom naproxen significantly increased the intensity of FDEIA symptoms.

A 34-year-old man with no significant personal or family history was admitted to our department because of 4 episodes of anaphylaxis during the previous 8 years. The patient reported rash and rhinorrhea after inhalation of cereals (haymaking, a visit to the mill). He regularly took very intensive exercise (distance running), which was always well tolerated.

Episode 1: On the day of the episode the patient ate muesli with milk for lunch. In the evening, after drinking 0.5 L of beer and dancing for 1 hour, he experienced watery nasal discharge and throat angioedema. These symptoms resolved with oral antihistamines.

Episode 2: Anaphylactic symptoms started about 10-15 minutes after ingestion of wheat beer. The patient had previously eaten chocolate cake and drunk coffee. Angioedema affected the face and throat and was accompanied by urticaria and rhinorrhea. Symptoms resolved slowly over an hour after taking oral antihistamines.

Episode 3: This was the most severe reaction. At 9:00 AM, the patient took a 250-mg naproxen tablet. His lunch contained wheat bran and rye. Four hours later he took a second 250-mg naproxen tablet, immediately before exercising. After 30 minutes of low-intensity physical activity, he experienced rash, edema (throat, mouth, and tongue), and respiratory distress. He took oral antihistamines, which brought no relief. Finally, he lost consciousness and was taken to the emergency department.

Episode 4: The patient ate muesli with nuts and milk for lunch and experienced angioedema, urticaria, and rhinorrhea

Table. Episodes of Food-Dependent Exercise-Induced Anaphylaxis

	Episode 1	Episode 2	Episode 3	Episode 4
Culprit food	Muesli with milk	Chocolate cake and coffee	Pancakes with wheat bran and rye	Muesli with nuts and milk
Culprit allergen (positive SPT)	Oat bran, wheat bran	Nuts, wheat	Wheat bran, rye	Nuts, oat bran, wheat bran
NSAIDs	–	–	Naproxen	–
Other factors	Beer	Wheat beer	–	–
Intensity of physical effort	Medium	No exercise	Low	High
Time from intake of culprit food to exercise	6 hours	–	4 hours	4 hours
Onset of anaphylaxis after exercise	1 hour	–	30 minutes	1 hour
Symptoms of anaphylaxis	Rhinorrhea, urticaria, throat angioedema	Throat and face angioedema, urticaria, rhinorrhea	Urticaria, throat angioedema, respiratory distress, loss of consciousness	Rhinorrhea, angioedema, urticaria

Abbreviations: NSAID, nonsteroidal anti-inflammatory drug; SPT, skin prick test.

during high-intensity exercise taken in the evening (running for 1 hour at a speed of 12-13 km/h). The symptoms resolved spontaneously after half an hour.

The episodes are summarized in the Table.

Skin testing was performed with standard series of aeroallergens and food allergens (Allergopharma, Reinbek, Germany), together with skin testing for cereal and nuts suspended in Coca solution without phenol (50% 0.9 NaCl and 50% glycerol) at a 5% concentration (vol/vol). Skin tests were positive to grain, grass pollen, rye, *Artemisia vulgaris* (mugwort), *Plantago major* (broadleaf plantain), *Urtica dioica* (common nettle), mold, *Cladosporium*, beef, hazelnut, peanut, citrus, wheat flour, orange, chamomile, oat bran, wheat bran, macadamia nut, cashew nut, and oatmeal. Total serum IgE was 174 IU/mL. Allergen-specific IgE was positive only to hazelnut (class 2). Specific IgE to wheat was negative and specific IgE to ω -5 gliadin and to high-molecular-weight glutenin were not determined.

An exercise test performed according to Anderton et al [8] revealed neither symptoms of anaphylaxis nor changes in spirometric values. Placebo-controlled oral provocation tests were performed with aspirin according to Nizankowska-Mogilnicka et al [9] and with naproxen (125 mg, 250 mg, 500 mg, each administered 1 hour after the previous dose). The results were negative with both drugs. We therefore made a diagnosis of FDEIA and informed the patient that he should avoid ingestion of specific food allergens together with NSAIDs or alcohol and for at least 6 hours before physical exercise.

Episodes 1, 3, and 4 followed a classic clinical course: ingestion of food allergen and postprandial exercise that resulted in anaphylaxis. Episode 2, which involved intake of wheat beer, seems to be the most interesting one. Wheat beer includes wheat, to which the patient is allergic, and alcohol, which is a known cofactor of FDEIA that induces increased food absorption from the gastrointestinal tract [10]. All of the FDEIA episodes were mild, except the third one, which was life-threatening. The most likely explanation for the severity of this event was the intake of naproxen shortly before physical exercise. To our knowledge, this is the first report of the role of naproxen in FDEIA. Other possible reasons for such a severe anaphylactic reaction were either allergy to naproxen or nonallergic hypersensitivity to NSAIDs, although the latter possibility was ruled out by the negative results in challenges with naproxen and aspirin. We believe that our results confirm the role of NSAIDs other than aspirin as cofactors of FDEIA. Consequently, we suggest rigorous avoidance of all NSAIDs, not only aspirin, taken simultaneously with the culprit food.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Maulitz RM, Pratt DS, Schocket AL. Exercise-induced anaphylactic reaction to shellfish. *J Allergy Clin Immunol.* 1979;63:433-4.
2. Kidd JM 3rd, Cohen SH, Sosman AJ, Fink JN. Food-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol.* 1983;71:407-11.
3. Harada S, Horikawa T, Ashida M, Kamo T, Nishioka E, Ichihashi M. Aspirin enhances the induction of type I allergic symptoms when combined with food and exercise in patients with food-dependent exercise-induced anaphylaxis. *Br J Dermatol.* 2001;145:336-9.
4. Kohno K, Matsuo H, Takahashi H, Niihara H, Chinuki Y, Kaneko S, Honjoh T, Horikawa T, Mihara S, Morita E. Serum gliadin monitoring extracts patients with false negative results in challenge tests for the diagnosis of wheat-dependent exercise-induced anaphylaxis. *Allergol Int.* 2013;62:229-38.
5. Yokooji T, Hamura K, Matsuo H. Intestinal absorption of lysozyme, an egg-white allergen, in rats: kinetics and effect of NSAIDs. *Biochem Biophys Res Commun.* 2013;438:61-5.
6. Takahashi A, Nakajima K, Ikeda M, Sano S, Kohno K, Morita E. Pre-treatment with misoprostol prevents food-dependent exercise-induced anaphylaxis (FDEIA). *Int J Dermatol.* 2011;50:237-8.
7. Matsuo H, Yokooji T, Morita H, Ooi M, Urata K, Ishii K, Takahagi S, Yanase Y, Hiragun T, Mihara S, Hide M. Aspirin augments Ige-Mediated histamine release from human peripheral basophils via Syk kinase activation. *Allergol Int.* 2013;62:503-11.
8. Anderton RC, Cuff MT, Frith PA, Cockcroft DW, Morse JL, Jones NL, Hargreave FE. Bronchial responsiveness to inhaled histamine and exercise. *J Allergy Clin Immunol* 1979; 63:435-440.
9. Nizankowska-Mogilnicka E, Bochenek G, Mastalerz L, Swierczyńska M, Picado C, Scadding G, Kowalski ML, Setkowicz M, Ring J, Brockow K, Bachert C, Wöhrl S, Dahlén B, Szczeklik A. EAACI/GA2LEN guideline: aspirin provocation tests for diagnosis of aspirin hypersensitivity. *Allergy.* 2007;62:1111-8.
10. Alcoceba Borràs E, Botey Faraudo E, Gaig Jané P, Bartolomé Zavala B. Alcohol-induced anaphylaxis to grapes. *Allergol Immunopathol (Madr).* 2007;35:159-61.

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