Collagenous Colitis in a Patient With Common Variable Immunodeficiency

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Palabras clave: Colitis colagenosa. Colitis microscópica. Budesonida. Inmunodeficiencia común variable. Autoinminudad.

Collagenous colitis is a poorly understood condition associated with autoimmunity that results in watery, nonbloody diarrhea [1]. Common variable immunodeficiency (CVID) is a heterogeneous group of antibody deficiency disorders resulting in recurrent infection, autoimmunity, malignancy, and inflammatory complications [2]. CVID is diagnosed when there is a marked reduction in both immunoglobulin G and at least 1 other immunoglobulin isotype and a failure to respond to vaccination after exclusion of other causes of hypogammaglobulinemia [3]. Gastrointestinal manifestations are common although there has been just 1 report of an atypical presentation of collagenous colitis in CVID [4]. We describe a second case of a patient with CVID and typical features of collagenous colitis in whom fecal calprotectin was used as an inflammatory marker. The condition was treated successfully with budesonide.

A 25-year-old woman was diagnosed with CVID in 2000 and commenced on intravenous immunoglobulin therapy after presenting with recurrent sinopulmonary infections since childhood. She had a history of intermittent nonbloody diarrhea from the 1990s, with a weight loss of 5 to 10 kg in 1996. Several nondiagnostic colonoscopies were performed during that time, but it was not until 2001 that collagenous colitis was found. The patient declined steroid treatment and received cholestyramine instead.

The diarrhea improved but in late 2004, the patient developed a flare-up, with motions occurring 8 to 12 times a day. This was associated with a weight loss of 5 kg over 3 months. Stool samples were negative for common pathogens, including *Giardia*. The patient was treated empirically with metronidazole, which made no difference. Inflammatory markers (C-reactive protein and erythrocyte sedimentation rate) were normal but fecal calprotectin was elevated at 395 µg/g (normal, < 60 µg/g). Colonic biopsies showed lymphoid hyperplasia without active inflammation, and this was thought to be consistent with the patient's CVID.

Fecal calprotectin had fallen spontaneously to 108 µg/g by this stage and as there was no evidence of inflammation, the patient was tried empirically on pancreatin (a mixture of lipase, protease and amylase) and various antibiotics, all of which were unhelpful. Her symptoms improved spontaneously in early 2006 but recurred towards the end of that year with diarrhea occurring more than 10 times a day. Fecal calprotectin levels rose to 252 µg/g and a repeat colonoscopy showed collagenous colitis (Figure).

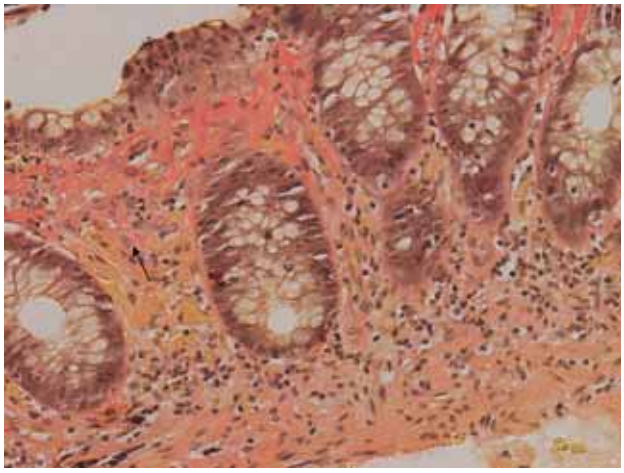

Figure. Collagenous colitis. A thickened subepithelial eosinophilic plate consistent with collagen deposition confirmed by Verhoeff-van Gieson staining for collagen.

The patient was commenced on 3 mg of budesonide 3 times a day in January 2007 and improvement was noticeable within 24 hours. Stool frequency reduced to 1 to 2 times per day and consistency also improved. Budesonide was tapered but the symptoms recurred when a dose of 3 mg once a day was reached. The patient currently requires a maintenance dose of 3 to 6 mg once a day and fecal calprotectin has fallen to 77 µg/g after 6 months of treatment.

The etiology of collagenous colitis is unclear. We postulate that it is an autoimmune phenomenon triggered by infection and/or drugs (such as nonsteroidal antiinflammatory drugs) given the association between collagenous colitis and autoimmunity, CVID, female preponderance [1] and a striking response to budesonide.

A Cochrane review concluded that budesonide improved disease status and quality of life in collagenous colitis [5].

This would suggest that conventional treatment for gut disease in CVID can be effective. The use of fecal calprotectin as an inexpensive, noninvasive marker is helpful in the diagnosis and monitoring of inflammatory bowel disease [6]. Furthermore, a study of collagenous colitis has shown that active disease is associated with elevated levels of this marker [7].

Gastrointestinal symptoms are common in CVID, but this is only the second report of collagenous colitis in CVID. It is unclear whether this might be due to underdiagnosis or a relative lack of awareness of the condition. Consequently, in patients with antibody deficiency and chronic diarrhea, a diagnosis of collagenous colitis should be considered, particularly given that effective treatment is available. This is the first report of the use of noninvasive fecal enzymes in a patient with CVID and bowel inflammation in whom systemic markers of inflammation were normal. The use of these tests should be further investigated in this setting.

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High Risk of Immediate-Type Reactions to Soy Drinks in 50 Patients With Birch Pollinosis

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Palabras clave: Abedul. Soja. Alergia alimentaria. Bet v 1. Gly m 4.

Soy products are often expected to have positive effects on human health; however, severe allergic reactions have been reported [1-3]. Soy contains 4 known allergens: 2 associated with soy hull through respiratory exposure and 2 associated with ingestion (profilin [Gly m 3], pathogenesis-related 10 family protein [Gly m 4]) [4]. Due to similarities between Gly m 4 and the birch pollen allergen, Bet v 1 [1,4,5], there is an increased risk of pollen-associated soy allergy in patients with birch pollinosis [1-3,5-8]. Previous investigations have shown that 71% (67/94) of subjects were cosensitized to Bet v 1 and to Gly m 4; 10% (9/94) were clinically relevant, with symptoms on ingestion of soy products [7]. We studied the frequency of cosensitization to Bet v 1 and Gly m 4 and its clinical relevance in patients in Germany.

Fifty consecutive patients diagnosed in our outpatient clinic with type I allergy to birch pollen (by positive skin prick test, specific immunoglobulin [Ig] E for birch, or both) completed a questionnaire on recent ingestion and tolerance of any soy products. In vitro tests included total IgE and specific IgE to soy (bean), birch, recombinant (r) Bet v 1, rBet v 2, rBet v 4, rGly m 4, and tryptase (Phadia, Freiburg, Germany; values ≥ CAP 1 assessed as positive). Data were evaluated by SPSS with significance at *P* values less than .05.

Mean total IgE was 318 kU_A/L, specific IgE was positive for rBet v 1 in 46 patients (92%), for rBet v 2 in 7 patients (14%), for rBet v 4 in 2 patients (4%), for rGly m 4 in 36 patients (72%, all of whom were also positive for rBet v 1), and for soy in 5 patients (10%). Tryptase was within the normal range in all patients. There was a significant correlation between the positivity of rBet v 1 and rGly m 4 (0.962; $P < .01$). Twenty-eight patients reported previous ingestion of soy products (soy drinks, n = 16; tofu, n = 7; soy sauce, n = 6; and others). It is noteworthy that 8 (50%) of the patients who reported ingestion of soy drinks had experienced clinical reactions (subgroup A) as follows: oral allergy syndrome (OAS, n = 2); OAS and angioedema (n = 3); angioedema and dyspnea (n = 1); angioedema, gastrointestinal, and cardiovascular symptoms (n = 1); and gastrointestinal symptoms (n = 1). None of them had reacted to any other soy food preparation. Seven of those patients were positive for rBet v 1 and rGly m 4, while 1 patient had a predominantly Gly m 4–independent soy allergy (positive skin prick test to birch, specific IgE, negative result

for rBet v 1, rGly m 4 coated allergen particle [CAP] class 1, soy CAP class 4).

There was no significant correlation between clinical symptoms on drinking soy products and specific IgE for Bet v 1 or Gly m 4. However, patients with clinical symptoms (subgroup A) tended to have higher Gly m 4 values than those without symptoms (subgroup B) (Table).

Table. Demographic Data and Specific Immunoglobulin E Values of the Patients Studied[a]

			Subgroup A	Subgroup B
	All	Soy drink ingestion	Adverse reaction after soy drink ingestion	No adverse reaction after soy drink ingestion
Number	50	16	8	8
Male: Female	20:30	9:7	4:4	5:3
Age, y	34 (23-45)	25 (20-40)	28 (20-40)	25 (21-34)
rBet v 1 –specific IgE, kU_A/L	13.5 (3-36)	19.7 (5-59)	28 (12-59)	26 (5-57)
rGly m 4 –specific IgE, kU_A/L	2.4 (0.5-8)	3.6 (1-13)	7 (3-17)	1.2 (0.9-9.1)

Abbreviations: Ig, immunoglobulin.
[a] Patients with clinical symptoms after soy drink ingestion (subgroup A, n = 8) tended to have higher Gly m 4 values than those without symptoms (n = 8; *P* = ns). Data are expressed as the median (interquartile range).

In soy allergy, the threshold dose of proteins leading to clinical symptoms has been identified as being significantly higher than in peanut allergy [9]. This is in line with our finding that clinical reactions appeared only after ingestion of soy drinks, that is, a fast intake of high amounts of protein [2]. Moreover, due to manufacturing processes, other soy foods such as tofu and soy sauce contain partially or extensively hydrolyzed protein with probably only minor amounts of Gly m 4, thus making them less allergenic.

In conclusion, 36 (72%) patients showed cross-reactive IgE to Bet v 1 and Gly m 4, thus confirming the observation by Ballmer-Weber et al [9]. Clinically relevant symptoms appeared only in patients who had ingested soy drinks, while other soy products were well tolerated. Thus, patients with Bet v 1 and Gly m 4 cross-reactivity should avoid drinking high amounts of nonhydrolyzed soy proteins.

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The authors declare no conflicts of interest.

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House-Dust Endotoxin Exposure and Recurrent Wheezing in Infants: A Cohort Study

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Palabras clave: Endotoxina. Polvo. Lactantes. Silbancias recurrentes.

Endotoxins are cell wall components with proinflammatory properties of the outer membrane of gram-negative bacteria. While house dust endotoxin concentrations have been associated with asthma severity in both adults and children [1], exposure to high endotoxin levels early in life may protect against the development of asthma and allergic disease [2,3]. Domestic endotoxin exposure is normally assessed using flooring and bedding reservoir dust [4]. We have previously shown that daycare centers in Brazil should be considered an important source of exposure to endotoxins [5].

The aim of this study was to investigate whether early endotoxin exposure might influence the development of recurrent wheezing in infants with a high risk of asthma. We also wished to investigate the association between reports of recurrent wheezing and potential predictive factors such as the presence of pets in the home, exposure to tobacco, a history of asthma in parents, eczema, and respiratory infections.

We enrolled 104 low-income newborns living in Paraisópolis, São Paulo, Brazil from January to March 2004 and followed them for 8 months. All the infants were at high risk of asthma (at least 1 parent had a history of allergy, asthma, or both). When the infants were 3, 6 and 8 months of age, their parents filled in a written questionnaire regarding their children's general health and the number of wheezing episodes they had experienced; recurrent wheezing was defined as at least 2 wheezing episodes in 6 months. Dust samples were collected from bedding and floors in the infants' homes and the daycare center (where the infants spent on average 8 hours a week) with a vacuum cleaner (1400W, airflow rate of 45 L/min) covering an area of 1 m^2 for 2 minutes. Fine dust was obtained by sieving samples through a mesh screen and diluting these in endotoxin-free water; all suspensions were diluted to at least 1:40 000. Endotoxin content was determined using the Kinetic Limulus Amebocyte Lysate assay (Pyrogent–5000, Cambrex Bioscience, Walhersville, USA). To prevent possible bias, a replicate of each sample was spiked with a standard endotoxin. When spike recovery was below 45%, the suspension was concentrated, and when it was over 200%, the suspension was diluted. The assays were run in duplicate and endotoxin levels were expressed as endotoxin units (EU)/mg of dust.

After 8 months of follow-up, 45 out of 104 infants (43%) presented recurrent wheezing but only 10 (9.6%) had been exposed to endotoxin levels of over 100 EU/mg from bedding. Bedding endotoxin levels ranged from 1.6 to 158 EU/mg (geometric mean, 21.4 EU/mg) while floor levels ranged from 1.6 to 2955 EU/mg (geometric mean, 18.7 EU/mg).

There were no significant differences between bedding or floor endotoxin levels in infants with and without recurrent wheezing. Endotoxin levels of over 100 EU/mg in the home (bedding and floor) decreased recurrent wheezing significantly (odds ratio [OR], 0.15; 95% confidence interval [CI], 0.03-0.7, $P < .01$), and similar results were observed with bedding endotoxin levels (OR, 0.13; 95% CI, 0.02–1.04; $P < .05$). In the group of infants with recurrent wheezing, only 2% (1/45) were exposed to endotoxin levels of over 100 EU/mg from bedding; 6% were exposed to the same endotoxin levels from the floor.

There were no significant differences between the groups in terms of reported recurrent wheezing and eczema, history of asthma in parents, exposure to environmental tobacco, or the presence of pets in the house. There was, however, a significant association between respiratory infections (present in 55 of the total group [n = 104] and 35 of the group with recurrent wheezing [n = 45]) and recurrent wheezing (OR, 6.9; 95% CI, 2.8-16.8; $P < .0001$). All the infants were exposed to endotoxins at the daycare center, where the mean endotoxin levels were 65.8 EU/mg in floor dust and 15.9 EU/mg in dust from mattress and chair surfaces.

High endotoxin exposure early in life might protect against the development of recurrent wheezing. Follow-up in these children will be important for the diagnosis of asthma.

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Selective Immediate Hypersensitivity to Etoricoxib

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Key words: Etoricoxib. Nonsteroidal anti-inflammatory agents. Hypersensitivity. Cross-reactivity.
Palabras clave: Etoricoxib. Antiinflamatorios no esteroideos. Hipersensibilidad. Reactividad cruzada.

Etoricoxib is a novel nonsteroidal anti-inflammatory drug (NSAID) that selectively inhibits the inducible form of cyclooxygenase (COX), COX-2. Etoricoxib has a higher COX-1:COX-2 selectivity ratio than other COX-2 selective NSAIDs such as rofecoxib, parecoxib, or valdecoxib. Etoricoxib is more than 100-fold selective for COX-2 than for COX-1 in whole blood assays, and has similar efficacy to traditional NSAIDs in rodent models of inflammation, pain, and fever, and also in a primate model of pyresis [1]. The drug comes in tablets of 60, 90, and 120 mg and is recommended for osteoarthritis, rheumatoid arthritis, acute gouty arthritis [2], toothache, and primary dysmenorrhea [3]. Etoricoxib and other COX-2 inhibitors have been tested in patients with a history of adverse reactions induced by NSAIDs and their tolerability was good [3].

We report the case of a 53-year-old man who was treated 2 months previously with etoricoxib due to chronic low back pain. Twenty minutes after the first dose, he presented generalized pruritus and urticaria. The following day he took another dose of etoricoxib and presented the same reaction, although it was more severe. Later, he tolerated acetaminophen, metamizole, and ibuprofen.

Skin prick testing with celecoxib and etoricoxib (10 mg diluted in 1 mL saline) was negative. Histamine was used as a positive control and normal saline as a negative control.

Single-blind placebo-controlled challenge tests with oral etoricoxib and celecoxib were performed on different days. Starting with 1/10 of the normal therapeutic dose, the doses were increased under strict monitoring at 45-minute intervals until the therapeutic dose was reached.

The patient tolerated celecoxib at the therapeutic dose, but 1 hour after the last dose (usual dose of 60 mg), he developed generalized itching and urticaria. The reaction disappeared within an hour with oral corticosteroids and antihistamines.

Cases of hypersensitivity to etoricoxib are rare. We found only 1 case of intolerance to NSAIDs with no tolerance to rofecoxib and etoricoxib [4] and 3 cases of etoricoxib allergy [5-7]. Atzori et al [5] reported a case of purpuric rash affecting the legs, thighs, and feet, and maculopapular lesions of the abdomen, trunk, and upper limbs after 12 days of treatment with etoricoxib due to chronic spondyloarthrosis. A skin biopsy was consistent with leukocytoclastic vasculitis. This eruption healed 2 weeks after etoricoxib was discontinued. Augustine et al [6] described a case of fixed drug eruption and generalized erythema occurring simultaneously after intake of etoricoxib. The diagnosis was confirmed by a patch test over the healed lesion caused by the fixed drug. Finally, Layton et al [7] report a case of Stevens-Johnson syndrome after 3 days of treatment with etoricoxib. However, this patient was receiving concomitant medication (alendronic acid, folic acid, dihydrocodeine, zopiclone, methotrexate, pyridoxine, and citalopram); therefore, it is difficult to say which drug led to the reaction.

COX-2 inhibitors belong to the diaryl heterocycles, which are characterized by a central carbocyclic or heterocyclic core that joins two aryl groups.

The structure of etoricoxib is somewhat different from that of other compounds, since one of the local phenyls is replaced by a pyridine. In addition, it also presents a pyridine as the central heterocyclic core [8] (Figure). This structural difference might be the reason for the selective sensitization to etoricoxib.

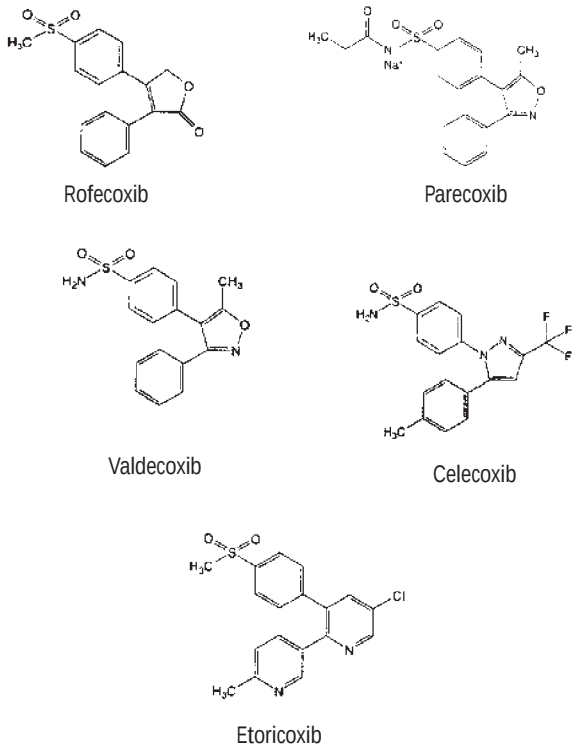

Figure. Chemical structures of COX-2 selective inhibitors.

In summary, we report a case of immediate urticaria due to etoricoxib confirmed by a challenge test. In our patient, the selective cutaneous response to etoricoxib and the tolerance to drugs of the same group such as rofecoxib and to other NSAIDs that are not COX-2 selective inhibitors suggest selective sensitization to etoricoxib.

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Allergic Rhinoconjunctivitis After Ingestion of Boiled Rice

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Key words: Food allergy. Immunoblot. Rhinoconjunctivitis. Rice.
Palabras clave: Alergia alimentaria. Inmunodetección. Rinoconjuntivitis. Arroz

Although rice (*Oryza sativa*) is one of the most widely produced and consumed cereals in the world, hypersensitivity reactions to this grain are uncommon. There have, however, been several reports of clinical symptoms associated with immunoglobulin (Ig) E mediated-reactions following contact with raw rice [1], inhalation of vapors from boiling rice [2], and ingestion of cooked rice [3,4].

We present the case of a 35-year-old woman without a history of atopy who presented at our allergy department due to 2 episodes of rhinoconjunctivitis involving sneezing, itching of the eyes, nose, and ears, and moderate nasal congestion; both episodes had developed within minutes of eating cooked rice. She had previously worked in a bakery for 5 years and had experienced similar symptoms when kneading rice flour; the problem was resolved when the flour was removed from the bakery. She had never experienced any symptoms when working with or after ingesting flour made from other cereals.

We performed skin prick tests using a battery of common inhalants, food allergen extracts, cereal flour extracts, storage mites, α-amylase inhibitor, and other enzymes (ALK-Abelló, Madrid, Spain). The only positive result was to rice flour (wheal diameter, 12 mm). Prick-to-prick tests performed with raw and boiled rice at different intervals (5, 10, 15 and 20 minutes) were all positive (wheal diameter, 6-7 mm).

Total serum IgE was 17.8 IU/mL, while rice-specific IgE was 1.32 kU/L (ImmunoCAP fluorescent enzyme immunoassay; Phadia AB, Uppsala, Sweden). Levels of IgE antibodies to the peach allergen Pru p3 and wheat α-amylase inhibitor were 0 kU/L, both measured using the ADVIA-Centaur platform (Bayer, Munich, Germany).

The patient immediately developed moderate rhinoconjunctivitis following an open, placebo-controlled food challenge test conducted with boiled rice; the symptoms were treated with oral and occular antihistamines.

Rice flour extract was prepared at 10% (w/v) in phosphate buffered saline (PBS) stirred for 90 minutes, and boiled rice extract was obtained by boiling rice seed in PBS at 10% (w/v) with continuous stirring for 30 minutes. After centrifugation, the supernatants were sterile filtered. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (15% gel) was performed under nonreducing conditions.

Once the extracted proteins had been separated, they were electrotransferred to nitrocellulose membranes [5]. The membranes were blocked with 5% bovine serum albumin in PBS for 1 hour at room temperature and incubated with a 1:5 dilution of patient serum followed by a 1:3000 mouse antihuman IgE monoclonal antibody dilution (HE-2) [6]. After the final incubation with a 1:5000 dilution of a rabbit antimouse IgG conjugated with peroxidase (RAM-HRP, Calbiochem, La Jolla, USA), enhanced chemiluminiscence (ECL; Amersham Biosciences, Piscataway, New Jersey, USA) conducted in accordance with the manufacturer's instructions to immunodetect IgE-binding proteins revealed a clearly defined band of 19 kDa (Figure). Blots containing transferred proteins were incubated with dilution buffer and pooled serum from nonallergic patients and used as negative controls.

Rice has been described as both a respiratory allergen [1] and a food allergen [4] but there are just 2 reports of an IgE-mediated reaction to cooked rice [3,4]. Both of these occurred in nonatopic patients with isolated allergies to rice and neither of the studies performed an immunologic evaluation. A variety of rice allergens have been described, such as the major rice pollen Ory s 1 (35 kDa) with a 66% sequence identity to the rye grass pollen allergen Lol p 1 [7]; a 14-16 kDa allergen in rice seed from the α-amylase/trypsin inhibitor family, responsible for occupational asthma in bakers [8]; a 33 kDa allergen with glyoxalase I activity [9], and a lipid transfer protein (LTP) [10]. Our patient had a negative SPT for grass pollen and an absence of in vitro sensitization to LTP or α-amylase. Cross-reactivity between different cereal grains has been demonstrated [11], but in our patient, all the SPT results for cereal flours other than rice flour were negative.

We have presented a new case of isolated rice allergy in a nonatopic patient who developed identical clinical symptoms

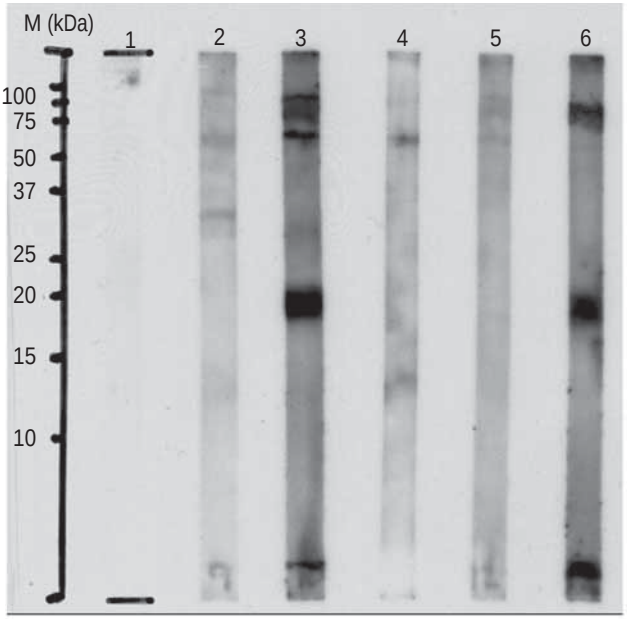

Figure. Immunoglobulin E detection. Rice flour extract (lanes 1-3) and boiled rice extract (lanes 4-6). Lanes 1 and 4, negative controls with dilution buffer; lanes 2 and 5, negative controls with pooled serum from nonallergic patients; lanes 3 and 6, patient serum. The molecular weight of prestained markers, run in parallel, is shown on the left.

following exposure to different forms of rice (raw and cooked) via different routes. We identified an IgE-binding band of 19 kDa in our analysis of both rice flour and boiled rice.

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An Unusual Clinical Presentation: Invasive *Candida* non-*albicans* Infections in Ataxia Telangiectasia

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Key words: Ataxia telangiectasia. *Candida* non-*albicans*
Palabras clave: Ataxia telangiectasia. *Candida* non-*albicans*

Ataxia-telangiectasia (AT) is an autosomal recessive disorder characterized by neurodegeneration and telangiectasia in the eyes and on the skin. Immunodeficiency and predisposition to cancer are common in patients suffering from this condition. The protein product of the gene mutated in ataxia-telangiectasia is activated in response to breaks in double-stranded DNA and has been shown to play a central role in the cellular response to radiation.

Immunodeficiency in AT patients is variable, and involves both humoral and cellular immune responses. However, despite laboratory evidence of significant immune system abnormalities, opportunistic infections are uncommon [1]. We present a case of AT, in which the patient developed unusual necrotizing lesions that were complicated by *Candida* non-*albicans* infection.

A 15-year-old girl was referred to our clinic with AT. She was a second child and her older sister had also been diagnosed with AT. She suffered from frequent infections of the upper and lower airways. One year ago skin ulcers appeared on her cheeks and disseminated verrucae on her hands. A depression began to develop on her nose and necrotizing lesions were visible in her nasopharynx. She was admitted to a dermatology

clinic and a skin biopsy revealed a granulomatous necrotizing inflammation. The results of acid-fast staining and the purified protein derivative test were negative. She was given interferon alfa-2 as treatment for her warts.

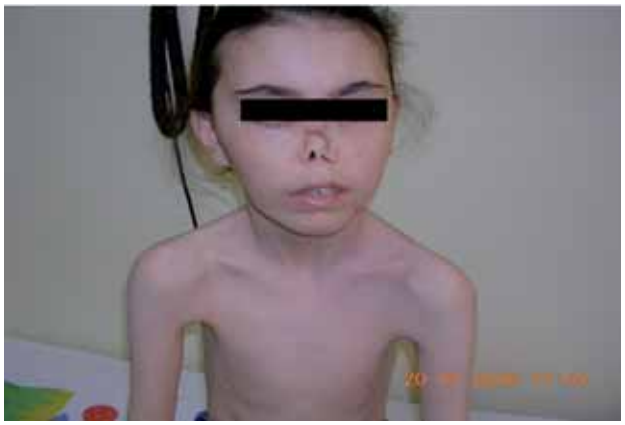

Figure 1. Depression on the nose due to disseminated necrotizing lesions in the nasopharynx.

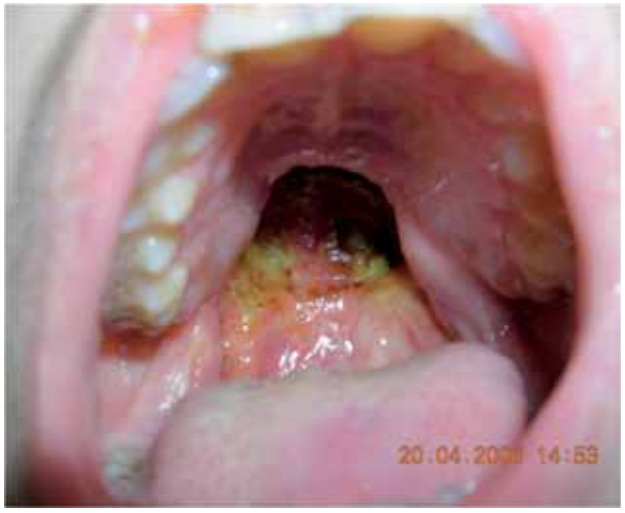

Figure 2. Necrotizing lesions in the nasopharynx.

Physical examination revealed growth retardation, severe ataxia, nystagmus, extensive necrotizing lesions in her nasopharynx, a depression on her nose (Figures 1 and 2), and a large number of warts on her hands.

An immunological evaluation revealed normal serum immunoglobulin (Ig) levels and isohemagglutinin titers together with $CD4^+$ cell lymphopenia and a severely diminished lymphocyte activation response to phytohemagglutinin.

A radiograph of the nasopharynx showed destruction of the bone in the nasal septum and hard palate. Computed tomography of the thorax revealed nodular infiltration and soft tissue densities in both lungs. These were thought to be due to aspiration of infected material from the nasopharynx.

Culture of a nasopharyngeal biopsy specimen yielded *Candida krusei* and *Candida glabrata*. The patient received intravenous liposomal amphotericin B for 3 weeks, after which time *C glabrata* was again isolated in a nasopharyngeal tissue swab culture. As the patient was resistant to amphotericin B, she received caspofungin for 25 days. She is still receiving treatment with itraconazole, trimethoprim-sulfamethoxazole, and intravenous immunoglobulin, and her clinical status has improved.

The incidence of infection in AT patients is quite variable. Frequent respiratory infections have been described in 83% of cases, and progressively destructive lesions are occasionally observed, especially in the lungs [1-5].

IgG, IgA, IgE, and IgG levels are generally reduced or absent in AT patients [2-4], and, although our patient had normal immunoglobulin levels, intravenous immunoglobulin replacement therapy was started to decrease the frequency and severity of the infections.

AT patients rarely acquire opportunistic infections, although they may be more susceptible to viral infections. Their T-cell function may be relatively intact, thus explaining the uncommon occurrence of opportunistic infections [6,7]. We suggest that the invasive infections in the case we report might be due to functionally impaired T cells.

There is much evidence to suggest that herpes simplex virus type 1 inactivates one of the repair mechanisms of double-stranded DNA breaks [8]. Therefore, the viral infection causing the verrucae may be responsible for the excessive humoral and cellular immunodeficiency observed in the case we report.

The patient had previously received interferon alfa-2 for her verrucae. Besides the antiviral effects of this drug, it has been shown to impair lymphocyte proliferation and macrophage activity [9,10].

Biological agents seem to be naturally resistant to conventional antifungal therapy, with the result that patients should be treated aggressively with the latest-generation antifungal drugs. Although it has been reported that opportunistic and severe viral infections are uncommon, existing viral infections or immunomodulatory treatments should be considered as additional factors leading to severe immunodeficiency.

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Effect of Omalizumab Treatment in a Baker With Occupational Asthma

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Key words: Occupational asthma. Anti-immunoglobulin E therapy. Omalizumab.

Palabras clave: Asma ocupacional. Tratamiento anti-IgE. Omalizumab.

Several studies have shown the clinical efficacy of anti-immunoglobulin (Ig) E therapy with omalizumab in severe persistent allergic asthma [1, 2], but potential applications in patients presenting occupational asthma have not been reported so far. A placebo-controlled study in care workers with occupational latex allergy showed a clinically relevant ocular and skin anti-allergic activity of omalizumab, although the study did not include any cases of moderate to severe asthma [3]. We describe a 1-year follow-up case study of a male baker with asthma treated with omalizumab.

A 35-year-old man weighing 96 kg revealed a 13-year history of perennial rhinitis and asthma. He had been working in a bakery, with exposure to high levels of airborne wheat flour particles, since the age of 20 years. During the past year, the patient had experienced 4 asthmatic exacerbations requiring emergency medical visits and multiple doses of oral prednisone. Respiratory function tests revealed a forced expiratory volume in 1 second (FEV_1 of 2.86 L (72.8% of predicted) and a FEV_1/forced vital capacity ratio of 64.4%. The bronchodilator test was positive, with a FEV_1 reversibility of 13%, and the fraction of exhaled nitric oxide (FeNO) (NioxMino; Aerocrine AB, Solna, Sweden) was 29 parts per billion (ppb). Skin prick tests (CBF-Leti, Barcelona, Spain) were positive for several kinds of flour (wheat, rye, barley and maize in a 10 mg/mL solution), mites (*Dermatophagoides farinae*, *Acarus siro*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, and *Glycyphagus domesticus*), and pollens (*Platanus*, *Olea*, and grass). Laboratory analysis (Pharmacia CAP System; Phadia AB, Uppsala, Sweden) revealed a serum IgE level of 817 IU/mL and positive serum-specific IgE to *Dermatophagoides farinae*, *Lepidoglyphus*, *Tyrophagus*, *Glycyphagus*, wheat, barley, rye, and maize. Fungal α-amylase sensitization was not analyzed by skin prick or specific IgE tests.

Specific immunotherapy was initiated using a long-acting depot extract of wheat flour (Bial Aristegui, Bilbao, Spain). Four hours after receiving a subcutaneous dose of 0.4 mL of a 0.71 mg/mL solution, the patient experienced a systemic reaction. Immunotherapy was subsequently discontinued and omalizumab (375 mg every 2 weeks) prescribed. Each time the patient returned to receive his dose of omalizumab, we recorded asthma medication requirements, emergency visits, spirometric parameters (FEV_1, peak expiratory flow [PEF]), and FeNO. Asthma-related quality of life questionnaire (AQLQ) scores, as determined by the Juniper questionnaire [4], were assessed before treatment and after 16 and 52 weeks. The skin prick and serum-specific IgE tests were repeated at 12 months.

When anti-IgE therapy was initiated, the patient was treated with oral prednisone (50 mg daily) and fluticasone (1000 µg daily) with salmeterol (100 µg daily) by inhalation. Prednisone was discontinued after 16 weeks and no further emergency visits were recorded. Spirometric determinations showed FEV_1 values of between 4.19 L and 2.92 L (variability, 24.1%), and PEF values of between 12.89 L/s and 8.94 L/s (variability, 30.3%). FeNO values ranged from 8 to 47 ppb.

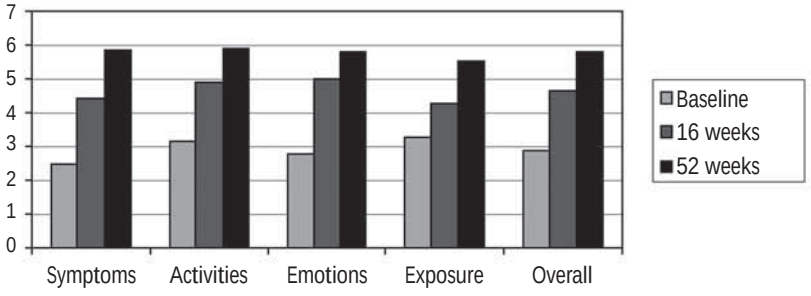

Figure. Asthma-related quality of life questionnaire AQLQ scores at baseline and 16 and 52 weeks of treatment with omalizumab.

Quality of life improvement was manifest in all domains of the AQLQ (Figure). The overall AQLQ score increased from 2.88 (before treatment) to 4.66 at week 16 and 5.81 at week 52. Skin prick tests repeated after 12 months were positive for grass pollens and *Tyrophagus*, and negative for other pollens, mites, and flour. The total serum IgE level after 12 months was 782 IU/mL, and specific IgE results for mites and flour were similar to baseline figures. The patient continued to work while receiving treatment with omalizumab.

The patient's clinical condition improved considerably, as was demonstrated by improved AQLQ scores, a reduction in the need for systemic corticosteroids, and fewer emergency visits. Changes in FEV_1, PEF, and FeNO, however, were irregular. Although skin responses to aeroallergens decreased after 1 year of follow-up, total and specific IgE serum levels remained similar, as previous studies have shown [5,6].

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Pomegranate-Dependent Exercise-Induced Anaphylaxis

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Key words: Exercise-induced anaphylaxis. Food allergy. Lipid transfer protein. Pomegranate.

Palabras clave: Anafilaxia inducida por ejercicio. Alergia a alimentos. Proteína de transferencia de lípidos. Granada.

Exercise-induced anaphylaxis (EIA) is characterized by the clinical features of systemic anaphylaxis after exercise. Food may be a precipitating factor of EIA (food-dependent EIA, [FDEIA]). Unlike nonspecific FDEIA, where the reaction is independent of the kind of food ingested before exercise, specific FDEIA is linked to the ingestion of specific foods before exercise. In this report we describe a patient with EIA triggered by ingestion of pomegranate.

A 17-year-old female patient reported an episode of widespread urticaria and lip edema during dance exercise. She was treated at the emergency room with intravenous corticosteroids and recovered in 1 hour.

The patient said that 3 hours earlier she had eaten some pomegranate seeds, with no other food. She underwent skin prick testing for the most common food allergens, and the results were positive for peach (wheal diameter 7 mm). The patient reported having had facial angioedema after ingestion of probiotics with peach flavoring during the previous year and after ingestion of fresh peach when she was young. Peach-specific immunoglobulin (Ig) E detected by direct enzyme-linked immunosorbent assay (ELISA) showed an optical density (OD) of 478 (negative control 156 OD).

Skin prick testing with fresh pomegranate seeds was positive (wheal diameter 6 mm), as was testing with peach lipid transfer proteins (LTPs) (wheal diameter 4 mm) (Lofarma SpA, Milan, Italy). The skin prick test for the most common inhalant allergens was negative. The patient has not eaten pomegranate since the reported reaction. We did not consider it safe to perform an oral challenge or a specific food-exercise challenge test with pomegranate because of the patient's clinical history and the temporal relationship between pomegranate ingestion, physical exercise, and onset of symptoms, in addition to the positive results of in vivo and in vitro testing for reaction to pomegranate. The absence of reactions after previous ingestion of pomegranate without exercise and the negative results of exercise challenge without ingestion of pomegranate strongly supported the diagnosis of specific FDEIA.

To confirm an IgE-mediated allergy to pomegranate and a possible involvement of LTPs, we performed a laboratory assay in which 100 g of fresh pomegranate was homogenized and extracted as previously described [1]. The protein content

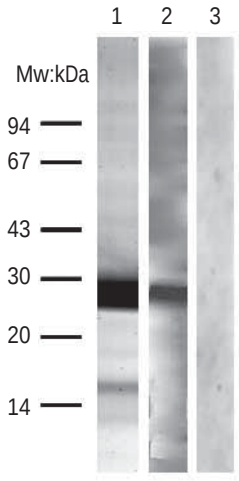

Figure. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis and immunoblot analysis. Lane 1, SDS-PAGE of pomegranate extract; Lane 2, Immunoglobulin (Ig) E reactivity of patient's serum; Lane 3, IgE reactivity of pool of nonatopic patients. Mw indicates molecular weight.

of the extract measured according to Bradford's method [2] (reagent from BioRad reagent, Milan, Italy) was 300 µg/mL.

Specific IgE, detected by direct ELISA, showed an OD of 346 (negative control 156 OD). The results of sodium dodecyl sulphate-polyacrylamide gel electrophoresis and immunoblotting are shown in the Figure. The patient's serum IgE reactivity to pomegranate extract was assessed by immunoblot analysis under reducing conditions. The extract was mixed with lithium dodecyl sulfate sample buffer (Nupage Bis-Tris, Novex, Prodotti Gianni, Milan, Italy) and 2-mercaptoethanol at 5% and heated at 100°C for 5 min before undergoing an electrophoretic run in a 10% polyacrylamide precast gel (Nupage Bis-Tris, Novex, Prodotti Gianni, Milan, Italy) at 180 mA for 1 h. The resolved proteins were transferred onto a nitrocellulose membrane for 1 h according to Towbin et al [3]. The membrane was saturated with 0.1 mol/L tris-buffered saline containing 5% fat-free milk powder (saturation buffer) and incubated for 16 h at 4°C with serum. After washing, specific IgE antibodies against pomegranate extract were detected by adding peroxidase-conjugated anti-human IgE antibodies from goat (Biospacific, Emeryville, California, USA; diluted 1:3500 in saturation buffer) using an enhanced chemiluminescence Western blotting substrate (Amersham, Milan, Italy).

Immunoblotting showed a component at about 30 kDa, similar to that of 29 kDa found by Gaig et al [4]. Experimental immunoblotting inhibition using peach LTP as an inhibitor was performed. No inhibition was observed (results not shown).

Pomegranate (*Punica granatum*) has been reported to cause IgE-mediated allergy [4,5] and immediate contact hypersensitivity [6], and there have been reports of cross-reactivity with nuts [7]. Moreover, a recent study showed that pomegranate contains 2 LTPs known as LTP1a and LTP1b [8].

Even if LTP allergens are involved in pomegranate allergy, the absence of an IgE-binding component of low molecular mass (7-9 kDa) characteristic of LTPs [8] confirmed the hypothesis that the 30-kDa component was involved in the allergic reaction experienced by our patient. This component was not related to LTPs.

To our knowledge, this is the first report of EIA after ingestion of pomegranate with the allergy confirmed by immunoblotting and no LTP cross-reactivity.

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The Basophil Activation Test in the Diagnosis and Follow-up of Hymenoptera Venom Allergy: An Alternative Point of View

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Key words: Allergy. Basophil. Flow cytometry. Hymenoptera.

Palabras clave: Alergia. Basófilo. Citometría de flujo. Himenópteros.

Recently, Dubois et al [1] reviewed the basophil activation test (BAT) in hymenoptera venom allergy. Those who are not familiar with the technique of flow-assisted analysis of in vitro activated basophils consider that the BAT has no established role in the diagnosis and management of insect sting allergy. Several technical and clinical issues merit reflection to complete the discussion of a technique that has been validated in approximately 750 patients and controls [2].

First, the authors state that the predictive value of the BAT in hymenoptera venom allergy is limited. In fact, the predictive value of the BAT is unknown, although the same holds true for venom skin tests (VST) and venom specific IgE (sIgE) tests. Moreover, the specificity of these tests is difficult to define, because it is impossible to determine beyond doubt the status of a stung individual who has never had a systemic reaction.

Second, the authors stress that the BAT has many pitfalls and address the issue of the heterogeneity of basophil responses and the effect of priming with interleukin (IL)-3. However, the heterogeneity of cell responses does not seem to be an issue [3,4]. CD63 expression is not restricted to a single allergen concentration, but rather it spreads over different log scales of stimulation concentrations, enabling experiments to be restricted to 1 or 2 optimal stimulation concentrations that discriminate between patients and controls. With respect to IL-3, this cytokine does not significantly influence maximum basophil activation by hymenoptera venom [5], although it might be deleterious in CD203c-based and p38 MAPK-based assays [6,7].

Third, the authors emphasize that the BAT fails to discriminate between systemic reactions and large local reactions. This is not surprising given the pathogenic mechanism of large local reactions. Over half the patients with large local reactions have positive sIgE and/or VST results. In fact, the criticism that the BAT does not discriminate between local and systemic reaction seems moot, since there is no rationale for testing patients with large local reactions [8].

Fourth, the authors speculate as to the use of the BAT in clinical decision-making in difficult cases, but state that this has not been conclusively demonstrated to date. We agree that, at the time of writing their review, there were no full papers available on this topic. However, we recently demonstrated [9] that diagnosis of hymenoptera venom anaphylaxis was not straightforward in 54 out of 118 patients due to discrepant (n = 47) or negative (n = 7) sIgE or VST results. When the BAT was single-positive for the suspected venom, it helped to establish diagnosis in 31 of the patients with discrepant sIgE and VST results and was diagnostic in the 7 patients with negative sIgE and VST results.

Finally, the authors state the BAT has no established role in the follow-up of venom immunotherapy (VIT). To date, 3 papers have addressed this issue and data are not consistent [3,4,10]. Two studies [4,10] show that BAT can monitor maintenance

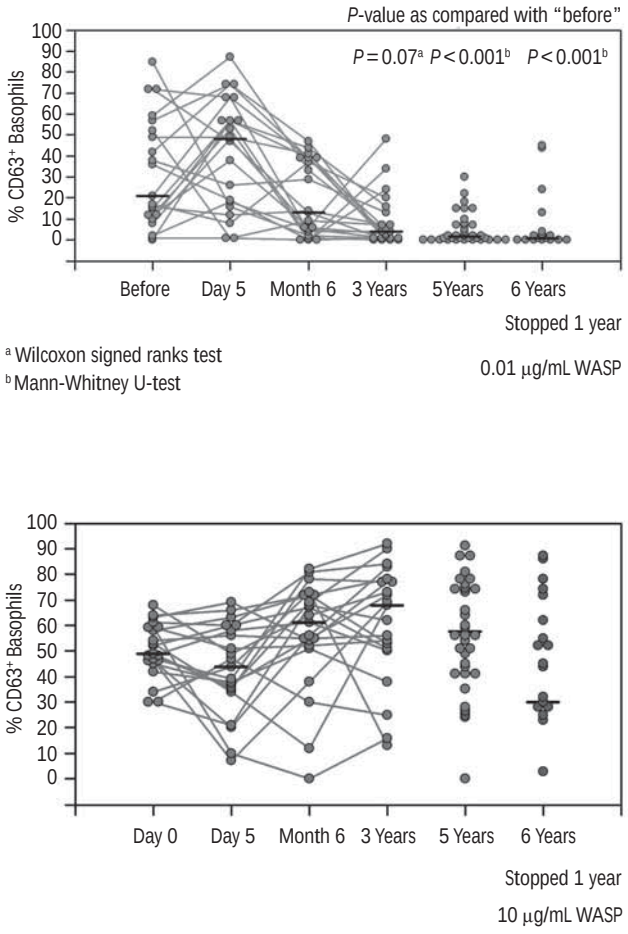

Figure. Effects of Wasp Venom Immunotherapy on the Sensitivity and Reactivity of Basophils.
Percentages of CD63-positive basophils in 21 wasp venom-allergic patients at baseline before treatment (day 0), after a semi-rush hyposensitization course (day 5), and after 6 months (month 6) and 3 years of maintenance therapy. Cross-sectional data at the end of treatment (5 years, n = 35) and 1 year after therapy was discontinued (n = 18). The upper and lower panels represent the data for basophil sensitivity (submaximal stimulation at 0.01 μg/mL) and basophil reactivity (maximal stimulation at 10 μg/mL), respectively. Bold lines denote the medians. These data are available from Ebo DG et al, Clinical Cytometry, part B, 2008.

VIT, provided the cells are stimulated submaximally. As summarized in the Figure, the observation that VIT decreases basophil sensitivity is confirmed in an extended analysis of 35 patients who stopped VIT after 5 years of treatment. Moreover, basophil sensitivity remains decreased after discontinuing VIT for 1 year.

In conclusion, although not all issues have been resolved, the BAT is a reliable additional tool for the diagnosis of hymenoptera venom allergy. With respect to the follow-up of VIT, there is evidence that treatment decreases the sensitivity of basophils and the BAT to the extent that therapy cannot be successfully monitored.

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Nonasthmatic Eosinophilic Bronchitis in a Baker Caused by Fungal α-Amylase and Wheat Flour

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Key words: Eosinophilic bronchitis. Induced sputum. α-amylase. Wheat flour.

Palabras clave: Bronquitis eosinofílica. Esputo inducido. Alfa-amilasa. Harina de trigo.

Nonasthmatic eosinophilic bronchitis is characterized by chronic cough, sputum eosinophilia, and absence of airway hyperresponsiveness to methacholine. Some cases of eosinophilic bronchitis are caused by work-related exposure to low- and high-molecular-weight allergens [1,2].

We describe the case of a 51-year-old nonsmoking woman who had been in charge of a bakery-patisserie for 14 years. She presented with an 8-year history of persistent chronic cough and tenacious sputum, but no wheezing or dyspnea. Her symptoms improved during holidays and nonworking days and worsened during workdays, especially when she was close to the bakery workroom. She had taken antitussive medication with no clinical improvement. Common causes of chronic cough, including gastroesophageal reflux, were ruled out.

The results of the physical examination were normal, as were the chest and sinus radiography findings. The absolute eosinophil count in peripheral blood was 100/μL. Skin prick tests were performed with wheat, rye, corn, barley, soy, oat flour extracts (5% w/v), fungal α-amylase (1 mg/mL), and common aeroallergens supplied by Bial-Aristegui (Bilbao, Spain) and ALK-Abelló (Madrid, Spain). Skin prick testing was positive for fungal α-amylase (12-mm wheal) and negative for the other allergens tested, including wheat flour. The total serum immunoglobulin (Ig) E level (Phadia CAP, Uppsala, Sweden) was 142 kU_A/L. Serum-specific IgE to fungal α-amylase was 3.83 kU_A/L, and to wheat flour 1.07 kU_A/L. IgE determinations to other cereal flours and baking additives were negative.

Spirometry revealed a forced vital capacity (FVC) of 3.7 L (106% predicted), a forced expiratory volume in 1 second (FEV_1) of 3.2 L (108% predicted) and a ratio of FEV_1 to FVC of 87%, with no significant changes after inhalation of salbutamol. Methacholine inhalation testing with the dosimeter method was performed on 3 separate occasions and did not reveal bronchial hyperresponsiveness (provocative concentration leading to a 20% fall in baseline FEV_1 > 16 mg/mL). Peak expiratory flow monitoring for 15 days at work and 15 days off work revealed

no significant daily variability. Specific inhalation challenge (SIC) was performed with fungal α-amylase and with wheat flour as previously described [3]. The patient developed dry cough during the challenges, but neither asthmatic reactions nor changes in methacholine responsiveness were observed 24 hours after the SIC.

Differential cell counts in induced sputum samples [4] showed 4.2% eosinophils when the patient was at work (within 24 h of an ordinary shift), and 1.1% after a period of 2 weeks away from work (baseline). SIC was performed again with α-amylase and 4 weeks later with wheat flour, and no asthmatic responses were observed. Results of the methacholine inhalation tests 24 h after the SICs were negative again. However, the differential cell count in induced sputum increased from less than 2% eosinophils to 33.3% 24 hours after SIC with α-amylase, and to 12.4% 24 hours after the SIC with wheat flour.

The patient was diagnosed with occupational eosinophilic bronchitis due to sensitization to fungal α-amylase and wheat flour. After 3 months of treatment with budesonide (800 μg daily) the cough subsided completely.

The causative agents of eosinophilic bronchitis in the workplace have rarely been established [1,2]. Challenge exposure to acrylate-containing glue in a worker at a company that produced weather strips for vehicles [1], to latex gloves in a nurse [2], and to wheat flour in a baker [5] resulted in a marked increase in sputum eosinophilia in the absence of both airflow obstruction and bronchial hyperresponsiveness. In addition, occupational eosinophilic bronchitis caused by low-molecular-weight agents such as diisocyanates [5], welding fumes [6], formaldehyde [6], and chloramine-T [7] has also been reported.

It is not known why patients with eosinophilic bronchitis do not have airway hyperresponsiveness despite the eosinophilic bronchial inflammation. A recent study suggests that the difference in airway function observed in subjects with eosinophilic bronchitis and asthma could be due to differences in prostaglandin E_2 production in the airways [8].

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ERRATUM:

"Study of Hypersensitivity Reactions and Anaphylaxis During Anesthesia in Spain"

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The title of Table 1 on page 351 should read "Drugs, Substances, and Concentrations Used in the Skin Test" and not "Patient Characteristics" as printed.