Premenstrual Asthma and Female Sex Hormones

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Palabras clave: Asma. Menstruación. Estradiol. Progesterona. Asma premenstrual.

Exacerbation of asthma during the premenstrual phase—premenstrual asthma (PMA)—affects more than 30% of women with asthma [1].

The causes of PMA are unclear, although the most prominent factors are associated with perimenstrual changes in estrogen and progesterone levels, as well as with modifications in the relationship between estradiol and progesterone [2,3]. However, findings are contradictory, and authors such as Pauli et al [4] found no association between asthma symptoms and serum estrogen and progesterone levels. Similarly, Pasaoglu et al [5] found no differences in estrogen and progesterone levels in asthmatic women with or without PMA.

Several studies have analyzed the effects of hormone therapy with estrogen, progesterone, or both in women with PMA [6]. Although results do differ occasionally, most studies show that the effects of hormone therapy are favorable.

We analyzed the role of female sex hormones in the pathogenesis of PMA. Measurement of sex hormone levels (and the ratio of one to the other in the preovulatory and premenstrual phases) and comparison between asthmatics with and without PMA can provide the basis for possible interventions.

Our methodology and partial results have been published previously [7]. We took blood samples to measure estrogens and progesterone levels during the preovulatory and premenstrual phases. Samples were frozen to –80ºC and analyzed at the laboratory of Hospital Juan Ramón Jiménez in Huelva, Spain using electrochemiluminescence immunoassay (E-170 autoanalyzer [Roche]). Given the sample size and the wide range of values for the hormones, data were analyzed using nonparametric methods (the Wilcoxon signed-rank test for matched analyses throughout the cycle and the Mann-Whitney test to compare changes between the groups with and without PMA).

We define PMA as premenstrual exacerbation of asthma characterized by a ≥20% increase in respiratory symptoms and/or ≥20% decrease in peak flow values with respect to the preovulatory phase [1]. Baseline patient characteristics (age, weight, and spirometry readings) were comparable in women with and without PMA. Blood samples in 59 asthmatic women of childbearing age were analyzed for both phases. A total of 31 women (52.5%; 95%CI, 39.8%-65.01%) were found to fulfill the criteria for PMA.

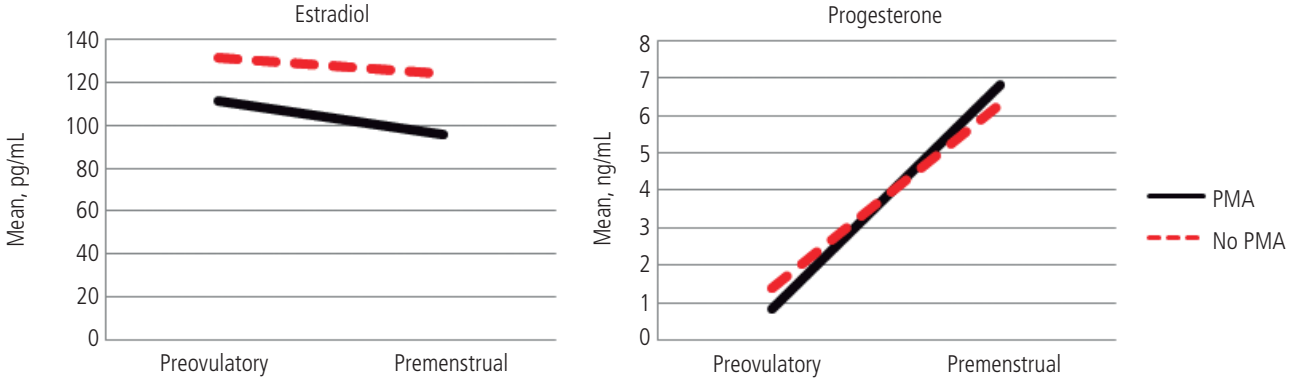

Figure. Hormone values during the preovulatory and premenstrual phases.

The Figure presents the hormone values for both phases. Women with PMA showed a slight premenstrual fall in estrogen levels (P=.677) and a clear increase in progesterone levels (P<.001). No significant differences were observed between the 2 phases in estrogen levels for women without PMA (P=.67), and progesterone values were higher in the premenstrual phase (P=.001).

Changes between the 2 phases in women with and without PMA were similar for estrogen levels (P=.845) and for progesterone levels (P=.225). The estrogen/progesterone ratio in both groups was similar in the preovulatory phase (P=.865) and premenstrual phase (P=.371).

The frequency of PMA did not increase with the severity of asthma: 6 women with PMA were mild intermittent (6/20, 30%), 9 mild persistent (9/12, 75%), 6 moderate persistent (6/10, 60%), and 10 severe persistent (10/17, 58.8%). No linear association was observed (P=.118), and denominators represented the total sample at each level of severity. In addition, hormonal changes between the preovulatory and premenstrual phases were similar for all 4 levels of severity (GINA 2005).

Female sex hormones appear to play an important role in respiratory illnesses such as bronchial asthma. However, as contradictory results in several studies have shown, the relationship is complex and relatively unclear.

Results from experimental models differ widely, and both inflammatory and anti-inflammatory effects have been described for estrogen and progesterone [8]. Changes in sex hormones in the menstrual cycle could also be related to inflammation of the airway in asthmatic patients [9,10]. Although the results are not uniform, estrogen may have a beneficial effect on asthma in women of childbearing age, whereas progesterone, whose levels are high in the luteal phase and practically nonexistent in the follicular phase, has a prejudicial effect on asthma.

The initial objective of our study was to discover whether asthma patients with PMA, as opposed to those without PMA, had decreased levels of estrogen or increased levels of progesterone in the premenstrual phase. However, our data did not confirm this hypothesis. Consistent with the findings of Pasaoglu et al [5], estrogen and progesterone levels did not differ significantly between asthmatic women with or without PMA. Likewise, their levels were within our reference range. Therefore, we believe that the real reason why some asthmatic women present with PMA might depend on other related factors and not on any clear changes in hormone levels during the menstrual cycle.

Our study is subject to a series of limitations. The 6-day preovulatory and premenstrual phases analyzed are perhaps too long to enable definitive conclusions to be drawn, and the fall in estrogen and progesterone levels during the premenstrual phase may not occur to the same extent in different women. In a previous study, we found the prevalence of PMA to be 43.7% (45/103) in the group sampled [1]. In the present study, the prevalence of PMA was 52.54% (31/59) in those women who consented to a blood test and 31.82% (14/44) in those who declined. The latter figure is somewhat lower (P=.058) than that of the women included in our analysis of hormone levels. The aim of the study was not to determine the prevalence of PMA, which could be affected by this possible selection bias, but to analyze the association between hormone levels and PMA, which would not be affected by this bias.

The results of our study cast doubt on the role of female sex hormones in the pathogenesis of PMA. Although our initial hypothesis that women with PMA had lower estrogen levels or higher progesterone levels in the premenstrual phase than women without PMA was an attractive notion, our results have not shown this to be the case.

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Anaphylaxis to Vapors of Roasting Chicken Controlled by Omalizumab

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Palabras clave: Alergia a carne de pollo. Alérgeno alimentario inhalativo. Anafilaxia. Omalizumab.

Anaphylaxis to the vapors of roasting poultry is uncommon [1,2]. We report a case of anaphylaxis to the vapors of roasting chicken that resolved after treatment with omalizumab. A 16-year-old girl consulted for several episodes of allergic reaction (about 10 episodes) following exposure to roasting poultry (chicken and turkey) during the previous 16 months. On at least 2 occasions, she presented laryngeal angioedema with dyspnea, wheeze, and bronchospasm for which she received inhaled adrenaline combined with systemic corticosteroids and emergency care. She had also experienced oral allergy syndrome after eating chicken during the previous 4 years and abdominal pain after eating turkey meat during the previous year. The patient had never been diagnosed with asthma and was not sensitized to any common respiratory allergens.

Skin prick tests were positive to fresh chicken and turkey meat, negative for cooked chicken and turkey meat, and negative for the remaining foods tested (spices, chicken liver, and eggs). Specific immunoglobulin (Ig) E testing was negative for chicken serum proteins, cat dander, mustard, beef, turkey meat, dog albumin, ovalbumin, and conalbumin (Phadia). Total serum IgE was 250 kU_A/L (Phadia). Tryptase levels were within normal values. A blinded challenge test was performed in an experimental chamber with chicken roasting in an oven. In order to ensure that the test was performed blind, the oven was hidden from the patient, who wore nasal clips to prevent the vapors from entering her nose. A clean empty functioning oven was used as a control. Wheezing, throat discomfort, and dizziness were recorded after 3 minutes of exposure; chest examination revealed bilateral wheezing and a 13% decrease in forced expiratory volume in 1 second. The patient was treated immediately with nebulized adrenaline and systemic corticosteroids. Blood pressure remained stable. Systemic tryptase levels were monitored before and after the test (2.4 g/L and 2.3 μg/L, respectively). No symptoms were recorded with the empty oven.

The patient (65 kg) was treated with omalizumab at a dose of 300 mg/mo. After the second injection, she no longer experienced symptoms upon exposure to the vapors of roasting chicken, for example, when she was close to the kitchen of her school. Eight months after the start of treatment, a second single-blind challenge test was performed under the same conditions. The patient did not experience any symptoms after 36 minutes of exposure to a chicken roasting in the oven. No

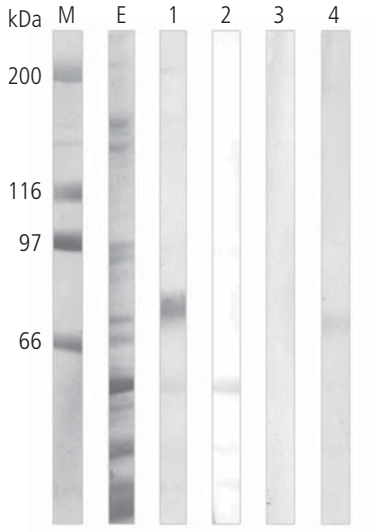

Figure. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (7.5%) of raw and cooked chicken meat extract. Lane M, molecular weight marker. Lane E, Coomassie staining reveals protein bands present in the raw chicken leg extract. Lane 1, the patient's IgE reacts with a 75-kDa band in the immunoblot. Lane 2, pooled sera of 5 nonallergic patients do not recognize the 75-kDa band. Lanes 3 and 4, chicken meat proteins heated for 20 minutes at 95°C. The 75-kDa protein is not recognized by the patient's IgE in the soluble protein fraction (lane 3) or in the precipitated protein fraction (lane 4).

change in lung function was recorded. Since then, the patient has been taking omalizumab at the same dose, although the dose was reduced to 300 mg every 6 weeks.

Protein extracts from raw and cooked chicken leg meat were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (Figure). IgE immunoblotting of the extract from native chicken leg with the patient's serum showed a band at 75 kDa that was not inhibited by chicken serum albumin, an allergen known to migrate within a similar molecular weight range. IgE reactivity to the 75-kDa band disappeared after cooking the extract for 20 minutes. The patient's serum showed no IgE reactivity when immunoblotted with roasted skin and roasted leg extract (data not shown). No IgE binding was detected in the solution eluted from the glass-fiber filter used to collect vapors (1 hour, 2.5 L/min) while the chicken was roasting in the oven.

Only 2 cases of severe asthma exacerbation after inhaling vapors of roasting chicken have been reported. In the first case, a young patient with allergy to chicken meat developed an asthma attack immediately after inhaling vapors while boiling hot dogs containing chicken [1]. In the second case, a young woman with allergy to chicken meat experienced symptoms of asthma after inhaling vapors from cooked chicken [2]. The results of skin tests to raw and cooked chicken meat were positive. IgE immunoblotting showed that she was not sensitized to chicken serum albumin but to meat proteins at molecular weights of 40, 25, 10, and 5 kDa. Our patient was sensitized to an unidentified 75-kDa protein, which was different from serum albumin and was affected by heat, thus confirming the patient's skin prick test results. We were not able to demonstrate IgE binding with the airborne material captured on glass-fiber filters. Omalizumab (Novartis) is a humanized anti-IgE monoclonal antibody, used for the treatment of persistent severe atopic asthma. Anti-IgE therapy has been studied in food anaphylaxis, mostly in peanut allergy, where it has been reported to increase tolerance to peanuts in allergic subjects [3]. However, only a few cases of anaphylaxis were controlled by omalizumab [4,5]. Anti-IgE therapy inhibits binding of specific IgE on the FcεRI receptor but also reduces cell surface expression of FcεRI on basophils and dendritic cells within 16 weeks [6]. This could explain the efficacy of omalizumab in our patient, whose condition improved within 3 months of treatment.

In conclusion, we report a case of moderate anaphylaxis induced by inhaling an airborne food allergen. The reaction was controlled with omalizumab. The suspected culprit allergen was an unidentified heat-labile 75-kDa protein that was different from serum albumin.

Conflicts of Interest

FDB has been a consultant for Stallergènes, Novartis, Mundipharma, and ALK-Abelló and has been a speaker and advisory committee member for MSD, Novartis, Stallergènes, and ALK laboratories.

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Cutaneous Allergy at the Supermarket

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Palabras clave: Cebolla. Calabacín. Alergia cutánea. Liliáceas. Cucurbitáceas.

Food is a common cause of allergy, and symptoms usually occur after ingestion. Shopping for food has never been considered a risk factor for allergic reaction.

We report the case of a 37-year-old woman who presented with bilateral ocular pruritus, redness, facial edema, and pharyngeal foreign body sensation. These symptoms developed while she was shopping at a supermarket. The episode began 5 minutes after handling onion and zucchini and persisted for the following 90 minutes until she received treatment with corticosteroids and antihistamines. She recovered completely 20 minutes after administration of treatment. The patient subsequently tolerated ingestion of both vegetables without developing a reaction, although she did not handle the vegetables. She had previously been diagnosed with allergy to grass pollen, peach, mustard, and nuts.

Fifteen days after the episode, the patient underwent skin prick tests with a complete food panel, a common inhalant allergen panel, and the panallergens lipid transfer protein (LTP) (0.1 mg/mL) and profilin (50 μg/mL) (ALK-Abelló). Positive results were obtained for grass pollen, peach, apple, hazelnut, chestnut, peanut, mustard, and LTP.

Prick by prick skin tests with onion and zucchini were positive (5 × 4 mm and 4 × 3 mm, respectively); the same tests were performed on 2 healthy controls, whose results were negative (prick-by-prick tests with onion produced a wheal of 1 × 1 mm in one of the controls and no wheals in the other; zucchini induced no reaction in either control). Patch testing with both vegetables was negative at 48 and 96 hours. Specific immunoglobulin (Ig) E to onion was 7.49 kU_A/L; specific IgE to zucchini was not determined owing to technical difficulties.

A specific challenge was performed with onion and zucchini on separate days. Given the nature of the first reaction and the lack of data in the literature, we performed a challenge test consisting of handling the vegetables with bare hands for periods of 30 seconds and 2, 5, 10, and 15 minutes, with a gap of 10 minutes between periods. The patient was asked to handle the vegetables as she would while shopping, although the vegetables did not come into contact with her eyes or her face. Both challenges were positive after a cumulative time of 3 minutes and 30 seconds, with periorbital edema and redness, tearing, and intense pruritus. Cutaneous redness was also observed on the hands.

Natural zucchini and onion proteins were extracted from chopped unpeeled vegetables by magnetic stirring (24 hours at 4ºC) in phosphate-buffered saline (pH 8) at 5% wt/vol. The extract was clarified by centrifugation (10 000 rpm for 20 minutes at 4ºC) and dialyzed against distilled water, before being filtered and freeze-dried. Protein concentration was estimated according to the method of Bradford [1]. Natural zucchini and onion extracts were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions. Protein bands (6 kDa to 97 kDa) were detected in both extracts using Colloidal Blue Coomassie (Sigma-Aldrich).

The extracts were loaded at 20 μg per lane for the immunodetection assays. The separated protein bands were transferred onto a nitrocellulose membrane (Bio-Rad) and blocked with 0.25% gelatin in NET Buffer (Tris HCL 0.5M, NaCl 1.5M, EDTA 0.05M, X-100 Triton 0.5%, pH 7.5) (BioRad). Membranes were incubated overnight with serum from the patient diluted 1:3 at 4ºC, then incubated with rabbit antihuman IgE (1:10 000) (DakoCytomation)–peroxidase conjugate, and detected using an enhanced chemiluminescence detection system (Amersham Biosciences) as recommended by the manufacturer. Several specific IgE-binding bands were obtained, with an apparent molecular weight ranging from 29 kDa to 70 kDa for onion extract and 40 kDa to 70 kDa for zucchini extract. Two protein bands at 45 kDa and 70 kDa were recognized by the IgE in both extracts (Figure).

Several studies have shown that LTP in onion [2,3] and profilin in zucchini [4] are associated with food allergy and cross-reactivity with pollen; however, our patient's serum did not recognize any of these bands. Asero et al [5] reported a 43-kDa protein to be a relevant allergen in onion; this band could be one of those recognized by our patient.

Cross-reactivity between onion and zucchini was investigated using immunoblotting inhibition assay. Preabsorption of serum with different amounts of zucchini extract (10 μg, 50 μg, 100 μg, and 200 μg) did not induce inhibition of IgE binding to immobilized onion extract, indicating that neither extract presented cross-reactivity.

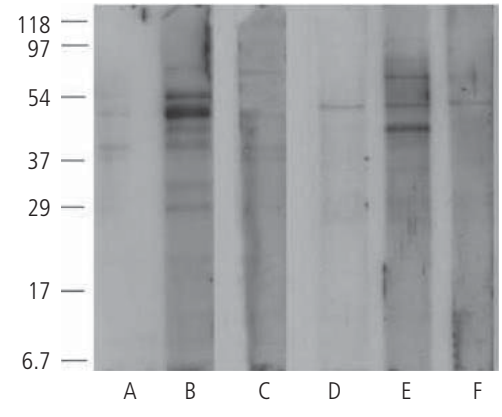

Figure. Immunoglobulin E immunoblot with onion (lane A, onion extract; lane B, patient's serum; lane C, negative control serum) and zucchini extract (lane D, zucchini extract; lane E, patient's serum; lane F, negative control serum).

In summary, we report the case of a patient with allergy to onion and zucchini in which an IgE-based mechanism was demonstrated, although we observed differences with published cases of allergy to these vegetables. Our patient had no symptoms after ingestion of cooked zucchini, in contrast with other studies [4]. Raw zucchini is not usually eaten in the patient's environment, and she refused to undergo testing with it. We believe these differences between positive skin and in vitro test results and negative oral challenge results could be due to the heat lability of the proteins involved in the allergic reaction, a finding that has already been described for onion by Arena et al [6]. Given the result of the skin and in vitro tests, it is difficult to explain why the patient tolerated both raw and cooked onion. Her reaction may have been caused by an unknown protein present only in the skin of the onions.

To the best of our knowledge, this is the first report of cutaneous symptoms caused by contact with zucchini.

Acknowledgments

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Peripheral CD8+ T-Cell Levels Are Decreased in Atopic Wheezing Children Aged Less Than 4 Years

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Key words: Asthma. Atopy. Wheezing. CD8+ T cells. Regulatory T cells.

Palabras clave: Asma. Atopia. Sibilancias. Células T CD8+. Células T reguladoras.

Epidemiologic studies indicate that the development of asthma and allergic sensitization is determined early in life; however, the immunologic mechanisms underlying these conditions have not been well studied. Abnormal distribution of T-cell subsets (eg, CD8+ T cells) has been associated with the severity of asthma in adults [1-4], and regulatory T cells (Treg) have been proposed as a key player in the pathogenesis of allergy and asthma [5].

The objective of our study was to assess quantitative differences in peripheral T-cell subsets, ie, CD8+ T cells and $CD4^+CD25^{hi}CD127^{lo}FoxP3^+$ Treg cells, between atopic infants at very high risk of developing asthma and healthy children.

Peripheral blood was obtained from 25 atopic children and 10 healthy controls aged <4 years to determine allergen-specific immunoglobulin (Ig) E (DPC Immulite 2000), total IgE, peripheral blood count, and T-cell subsets. The inclusion criteria for atopic children were history of >1 wheezing episode, positive skin test or specific sIgE result (*Dermatophagoides pteronyssinus*, cat hair, cockroach, mouse, dog, tree, grass, or ragweed), eczema, or parental history of asthma. The healthy control population had no clinical signs of asthma or allergy and no first-degree relatives with asthma or allergy symptoms. Parents reported compliance with asthma control medication for at least 2 weeks prior to blood sampling. Peripheral blood mononuclear cells were stained with anti-FoxP3 (eBioscience), anti-CD127 and anti-CD3 (Beckman Coulter), and anti-CD4, anti-CD8, and anti-CD25 (BD Pharmingen). The cells were acquired on a 4-laser FACS LSR-II instrument (BD Bioscience) and analyzed using FlowJo cytometric software (Treestar). The study was approved by the Institutional Review Board of the Albert Einstein College of Medicine, and all parents provided written informed consent.

No differences were recorded between atopic wheezers and healthy controls in terms of mean (SD) age (31 [10] months vs 35 [10] months; *P*=.281), gender (60% vs 80% male, *P*=.393), or ethnicity (in both groups 90% of children were Hispanic and African American). Atopic wheezers had a mean of 9.6 (8.5) wheezing episodes prior to enrolment, and 86% had a history of eczema. Atopic wheezers had significantly higher total sIgE levels then healthy children (684.6 [708.5] IU/mL vs 34.0 [57.3]; *P*=.007) and were sensitized to a mean of 2.6 (2.0)

aeroallergens and 3.4 (2.4) food allergens. Serology testing revealed no allergic sensitizations in the healthy controls.

In comparison to healthy children, atopic wheezers had significantly more white blood cells (WBC), mainly owing to greater numbers of granulocytes (2065 [551]/μL vs 3452 [1752]/μL, *P*=.02) and eosinophils. In addition, atopic wheezers had significantly lower percentages of $CD8^+$ T cells within the $CD3^+$ lymphocyte population (Figure). No statistically significant differences in Treg counts were recorded between healthy children and atopic wheezers.

A stratified analysis was made to compare atopic wheezers not taking corticosteroids or montelukast (n=7), atopic wheezers taking inhaled corticosteroids but not montelukast (n=8), and atopic wheezers taking inhaled corticosteroids and montelukast (n=7). Potential confounders (ie, age, sex, ethnicity, number of wheezing episodes, asthma hospitalizations, history of eczema, and exacerbations) were equally distributed among the 3 comparison groups (*P*>.15).

Atopic wheezers not taking asthma control medication had significantly lower absolute $CD3^+$, $CD4^+$, and $CD8^+$ T-cell percentages and a higher CD4:CD8 ratio than atopic wheezers on controller medication. The use of montelukast in addition to inhaled corticosteroids was associated with a further decrease in the CD4:CD8 ratio (*P*=.006) (Figure).

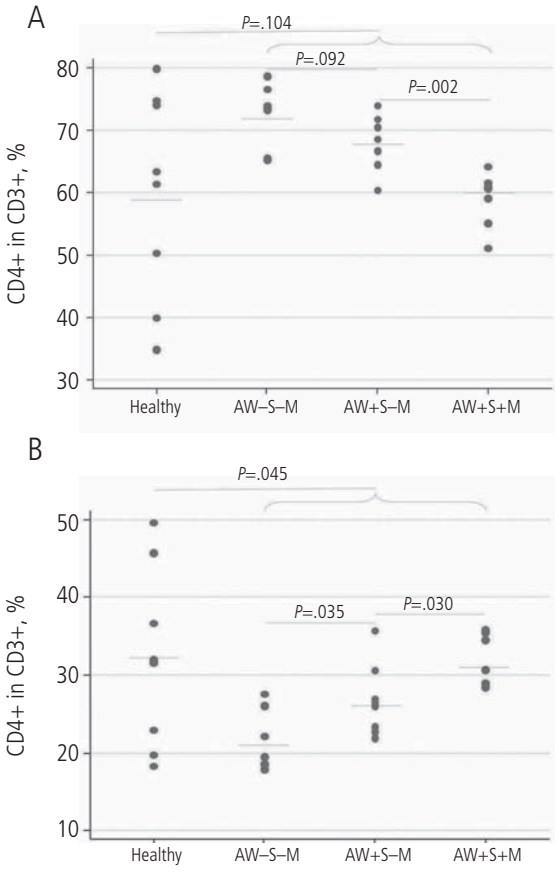

Figure. Frequency of peripheral $CD4^+$ T cells (A) and $CD8^+$ T cells (B) AW–S–M indicates atopic wheezers not taking inhaled corticosteroids or montelukast; AW+S–M, atopic wheezers taking inhaled corticosteroids but not montelukast; AW+S+M, atopic wheezers taking both inhaled corticosteroids and montelukast.

Our study demonstrated a decreased peripheral $CD8^+$ percentage and increased $CD4^+$ percentage in infants at very high risk of developing asthma compared to healthy controls and partial normalization of percentages of $CD8^+$ and $CD4^+$ in association with the use of asthma controller medication. Consistent with other studies, we found no differences in Treg cell counts between atopic and healthy infants [5,6]. While decreased peripheral $CD8^+$ cell counts have been described in older asthmatics, to our knowledge, this is the first report of such findings in children <4 years old who are at high risk for persistent asthma. In addition, the effect of inhaled corticosteroids and montelukast on peripheral T-cell subsets has not previously been established in young children.

Decreased peripheral blood $CD8^+$ T-cell counts in older children and adults with asthma, regardless of atopic status [3], could result from recruitment of $CD8^+$ cells into the airways as a result of airway inflammation [4]. Bratke at al [2] demonstrated lower levels of $CD8^+$ T cells expressing granzyme A, granzyme B, and perforin in the peripheral blood of asthmatics than in nonasthmatics [2]. In contrast, Machura et al [7,8] found no differences in $CD8^+$ and $CD4^+$ T-cell subsets between asthmatics and healthy children aged 3-18 years. However, most of the asthmatic children used inhaled corticosteroids, possibly attenuating the effect of asthma on peripheral T-cell subset variations, as supported by our observations.

Corticosteroids can induce $CD8^+$ T-cell populations that synthesize high levels of interleukin (IL) 10, while at the same time greatly reducing levels of disease-promoting IL-4 and IL-5 [9]; this finding could explain the increased $CD8^+$ frequency we observed in children taking inhaled corticosteroids. Alternatively, control of airway inflammation alone may help to normalize the peripheral imbalance between $CD4^+$ and $CD8^+$ cells, as shown in the study by Lee et al [4], where the peripheral CD4:CD8 ratio was increased during an asthma exacerbation but decreased after treatment with inhaled $ß_2$-agonists (and no corticosteroids) for 2 weeks.

Our findings help to clarify the peripheral immune pathogenic phenotype associated with a high-risk asthmatic clinical phenotype in early childhood. In addition, our results suggest that peripheral T-cell subsets can be altered in response to asthma control medication, indicating a confounding effect that should be taken into account in similar studies. Differentiation of $CD8^+$ subsets (ie, memory vs cytotoxic) in young atopic infants will be the next step towards clarifying the role of $CD8^+$ and $CD4^+$ T-cell subsets in the development of asthma.

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Generalized Skin Lesions in a Patient With Common Variable Immunodeficiency

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Key words: Common variable immunodeficiency. Skin granuloma. Anti-TNF-α.

Palabras clave: AInmunodeficiencia común variable. Granuloma cutáneo. Anti-TNF-α.

Common variable immunodeficiency (CVID) is a congenital disease that encompasses a wide range of immunological disorders characterized by antibody deficiency [1]. Granulomatous lesions have been reported in many body organs, although cutaneous granulomas are uncommon in patients with CVID [2-4].

A 36-year-old woman with CVID was referred to our Dermatology Department because of generalized cutaneous lesions. She had been diagnosed with CVID at the age of 16 after recurrent gastrointestinal and bronchopulmonary infections. The patient had also had seronegative arthritis since she was 20 years old. At the time of consultation, she was receiving parenteral immunoglobulin (400 mg/kg every 3 weeks), methotrexate (5 mg twice a week), and leflunomide (20 mg daily) for her arthritis.

During the previous 3 years, cutaneous lesions had appeared progressively on the upper extremities and spread to the lower extremities, face, and trunk. The lesions were asymptomatic, and no other clinical symptoms were reported. Physical examination revealed infiltrative erythematous-violaceous papules that coalesced into larger plaques with superficial telangiectasia (Figure, A). The lesions healed spontaneously as atrophic yellowish plaques.

Immunological tests revealed a profoundly reduced serum immunoglobulin (Ig) G concentration (<100 mg/dL) and a functional defect in IgG responses to immunization. The white blood cell count and serum concentrations of IgM, IgA, and IgE were normal. The results of T- and B-cell immunophenotyping (naïve B cells, nonswitched memory B cells, and switched memory B cells) were also normal.

Five biopsy specimens were obtained from the erythematous-violaceous papules of the right arm (2 for histologic examination and 3 for culture [bacteria, fungi, and mycobacteria]). All cultures were negative, and histopathological examination showed cutaneous granulomas with no caseous necrosis located in the deep dermis and superficial subcutis and surrounded by a severe lymphoplasmacytic infiltrate and isolated multinuclear cells (Figure, B). No mucin accumulation was observed, and no microorganisms were identified on specific stains (periodic acid–Schiff, Grocott), not even for acid-fast bacilli.

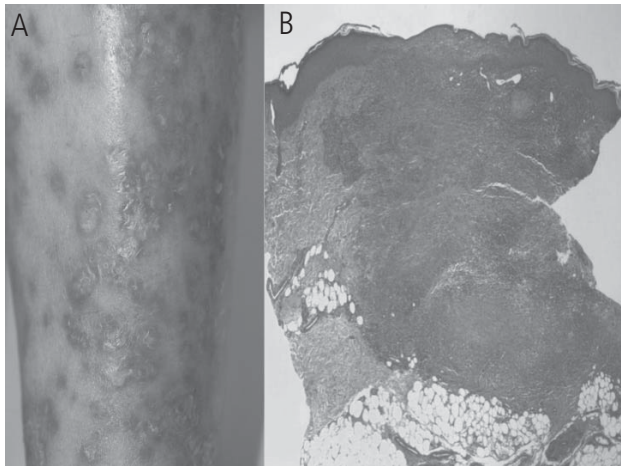

Figure. A. Infiltrative erythematous-violaceous papules coalesce into larger plaques with superficial telangiectasia on the right leg.
B. Cutaneous granulomas located in the deep dermis and the superficial subcutis with central noncaseous necrosis surrounded by severe lymphoplasmacytic infiltrate and isolated multinuclear cells (hematoxylin-eosin, original magnification ×10).

Further investigations revealed that the values for complete blood count, C-reactive protein, and angiotensin-converting enzyme were within the normal range. The result of the tuberculin skin test (5 TU) was negative on 2 occasions. High-resolution computed tomography scans of the thorax and abdomen showed bilateral basal bronchiectasis and homogeneous splenomegaly, both of which were long-standing and had remained unchanged over the last previous 10 years.

Consequently, the patient was diagnosed with granulomatous dermatitis secondary to CVID. Treatment with potent local corticosteroids under occlusion and oral corticosteroids (1 mg/kg prednisone daily), josamycin (500 mg daily for 3 months), and doxycycline (100 mg daily for 3 months) was unsuccessful. In order to treat not only the cutaneous granulomas, but also the seronegative arthritis, the patient started treatment with etanercept (25 mg subcutaneously twice a week), with a significant improvement in both cutaneous and articular symptoms.

CVID encompasses a wide range of immunological disorders characterized by antibody deficiency [1], which can be accompanied by functional impartment of T cells [1]. CVID is the second most frequent congenital immunodeficiency, although no robust data are available on its prevalence, which is estimated at 1 case per 50 000 newborns per year [1]. The disease generally presents in adulthood; however, earlier diagnosis is increasingly common [1].

The most common disorders affecting patients with CVID are repeated bacterial infection, autoimmune diseases, malignancy, and granulomatous disorders, which have been described in many cases of immunodeficiency [5].

Chronic granulomatous disease is the most common immunodeficiency associated with granulomas [5] and affects between 8% and 22% of CVID patients. In addition, patients with granulomas are thought to be more prone to autoimmune phenomena [1], as was the case in our patient (seronegative arthritis).

Granulomatous lesions have been reported in many organs, mainly the liver and lung, and in the gastrointestinal tract, although they have rarely been reported in skin [2-4]. The lesions take the form of erythematous or reddish-purple papules, plaques, nodules that are often ulcerated, or atrophic plaques with superficial telangiectasia [3,5]. They are usually located on the upper extremities but have also been described on the face and buttocks [6].

The 3 types of granulomas are nonnecrotizing epithelioid granuloma (sarcoid-like), caseous granuloma (tuberculoid), and necrobiotic granuloma [5]. Our patient had necrobiotic granulomas. As granulomatous skin lesions in patients with CVID can show histological features suggestive of infective granulomas and sarcoid-like and necrobiotic granulomatous disorders, those conditions have to be ruled out by additional tests.

Pathogenesis is unknown. Many authors propose that the presence of granulomas not only requires antibody deficiency, but also alteration of cellular immunity [5]. However, our patient's immunology test revealed normal T-cell and B-cell functions. An antigenic stimulus (nonidentified microorganism or environmental antigen) is also thought to cause the development of these lesions [7]. Furthermore, dysregulation of cytokine function has been associated with the development of granulomas [8]. Tumor necrosis factor (TNF) α is the main cytokine implicated in this process, and patients with CVID may have abnormal TNF-α synthesis [9]. TNF-α is a pleiotropic inflammatory cytokine, which is an essential mediator in inflammation and immune regulation, for example, in the proliferation of monocytes and macrophages. Many authors propose that persistent activation of TNF-α can increase the risk of granuloma formation in CVID patients [6,9].

Treatment with anti–TNF-α agents (etanercept and infliximab) has proven successful when indicated [6,9,10]. Therefore, we decided to try etanercept (after rigorous testing to rule out infectious disease), which proved to be an appropriate treatment for the cutaneous lesions and seronegative arthritis. Our patient did not present any side effects after 6 months of treatment.

To conclude, patients with CVID and cutaneous lesions are a challenge for clinicians.

Granulomatous dermatitis associated with CVID is infrequent and should be treated by a multidisciplinary team (dermatologists, internists, rheumatologists, and immunologists). Abnormal TNF-α synthesis appears to play a leading role in the development of granulomas in CVID patients. Consequently, anti–TNF-α agents are emerging as a new therapy for patients with CVID granulomas in whom previous infectious disease has been ruled out.

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Delayed Anaphylactic Reaction to Mammalian Meat

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Key words: Anaphylaxis. Food allergy. Mammalian meat. Cetuximab.

Palabras clave: Anafilaxia. Alergia alimentaria. Carne de mamíferos. Cetuximab.

Mammalian meat allergy is relatively rare in adults. Food allergies are type I immediate hypersensitivity reactions [1], which are immunoglobulin (Ig) E–mediated and occur within 5 to 30 minutes after oral intake of an offending food, but may occasionally occur up to 2 hours later [2].

We report the case of a 74-year-old woman with no history of atopy of allergic disorders who developed anaphylaxis 5 hours after eating mammalian meat. Two months after being bitten by ticks 3 years ago, she experienced delayed anaphylaxis following ingestion roast beef. Thereafter, she avoided beef, pork, lamb, horsemeat, chicken, and turkey. She also developed anaphylaxis 2 hours after drinking a glass of cow's milk. An allergy workup at our hospital using ImmunoCAP (Phadia) revealed levels of serum specific IgE antibodies against beef (2.46 kU_A/mL), pork (1.51 kU_A/mL), mutton (2.61 kU_A/mL), and milk (1.52 kU_A/mL). We performed a skin prick test (SPT) on the volar surface of her arm using commercial allergens (Torii Pharmaceutical Co: 1:20 wt/vol for milk and 1:10 wt/vol for wheat, soy bean, rice, salmon, cod, tuna, horse mackerel, and saury). Dihydrochloride histamine (10 mg/mL) was used as a positive control, and 0.9% saline solution was used as a negative control. The patient reacted only to milk and dihydrochloride histamine. We then performed SPTs using fresh milk. The patient reacted to fresh milk and dihydrochloride histamine. We performed an open oral challenge oral using chicken and pork. The patient ate 50 g each of roast chicken and roast pork without seasoning. She did not develop allergic reactions after eating chicken. However, she developed swelling and redness of the face and generalized wheals 5 hours after eating pork.

Patients with mammalian meat–induced anaphylaxis show high levels of IgE antibody against cetuximab. This antibody is specific for the oligosaccharide galactose-α-1,3-galactose (α-gal), which is present in the Fab portion of the cetuximab heavy chain [3]. The IgE antibody against α-gal is specific for a carbohydrate common to mammals, but absent from poultry and fish [3]. Pork-cat syndrome is another type of mammalian meat–induced anaphylaxis that is caused by cross-reactivity between cat albumin and pork albumin [4]. Therefore, patients with pork-cat syndrome have high levels of IgE antibodies against Fel d 1 and Fel d 2 [4].

We measured levels of specific IgE to Fel d 1 and Fel d 2 in the serum of our patient using the CAP-FEIA system

(Phadia). Colorimetric enzyme-linked immunosorbent assay was also carried out to determine the level of IgE antibodies to cetuximab coupled to cyanogen bromide–activated paper disks (100 μg/disk), as previously described [5]. The assay was calibrated using a control curve obtained with disks coupled to Japanese cedar pollen extracts and serial dilutions of pooled serum from patients with Japanese cedar pollen allergy; the level of the IgE antibody in these patients was measured in advance using the CAP system. The results were extrapolated from the control curve and expressed as units/mL. An IgE antibody level of ≥0.35 units/mL was regarded as positive. The specific IgE antibody levels were as follows: Fel d 1, <0.35 units/mL; Fel d 2, 0.53 units/mL; cetuximab, 14.8 units/mL. These levels were high for cetuximab and low for Fel d 1 and Fel d 2. Nuñez et al [6] reported similar results in European patients with delayed mammalian meat–induced anaphylaxis. Our patient developed anaphylaxis only after ingesting mammalian meat and milk. Most allergic reactions to meat are reported to occur within 2 hours after intake [7]. However, Commins et al [3] reported a case of delayed anaphylactic reaction to mammalian meat. Their diagnosis was based on a detailed interview with the patient and an SPT using fresh meat. They did not perform an open oral challenge. Our patient had a history of delayed anaphylactic reaction to beef. We confirmed a delayed anaphylactic reaction to mammalian meat on the basis of the results of the open oral challenge with pork. The allergic reaction was not observed after avoiding foods containing mammalian meat. Commins et al suggested that tick bites may have triggered the production of the IgE antibody against α-gal. Levels of IgE antibody against α-gal following tick bites have been reported to increase by at least 20-fold [7]. Ingestion of food containing α-gal does not induce sensitization against α-gal [3]. Our patient also had a history of tick bite before her first episode of anaphylaxis. Therefore, we considered that her history of tick bite had triggered the production of the IgE antibody against α-gal.

Moreover, our patient also experienced anaphylaxis after ingestion of milk, possibly owing to the presence of α-gal in milk. Indeed, IgE-inhibition analysis using serum from our patient showed that IgE reactivity to cetuximab was 87% inhibited by preincubating the milk, thus indicating the presence of cross-reactive α-gal in the milk. However, the clinical relevance of a positive SPT result or positive IgE to milk among α-gal–sensitized meat-allergic patients remains open to debate. Commins et al [3] showed that, although 20 of 24 α-gal–sensitized patients with mammalian meat–induced anaphylaxis showed increased levels of IgE antibody to milk, only 10 reported symptoms of allergy to milk [3]. Further research is needed to determine the relationship between the IgE antibody to α-gal and clinical reactivity to ingested milk.

Food allergy must be diagnosed accurately, since allergic reactions can recur. Meat is an important source of protein that is difficult to avoid. Patients with meat allergy should not try to avoid all kinds of meat. Therefore, in the case of a diagnosis of mammalian meat–induced anaphylaxis, it is important to remember that patients can tolerate chicken, turkey, and fish.

The workup for patients with beef or pork allergy should include testing for allergy to all kinds of mammalian meat and cetuximab. We report the first case of mammalian meat–induced anaphylaxis outside the United States and Europe that was confirmed by open oral challenge to determine levels of IgE against cetuximab. This type of anaphylaxis could affect patients worldwide.

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Rapid Desensitization With Temozolomide in Patients With Delayed Maculopapular Rash

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Key words: Temozolomide. Maculopapular lesions. Desensitization. Delayed allergic reaction.

Palabras clave: Temozolomida. Lesión maculopapulosa. Desensibilización. Reacción alérgica tardia.

Delayed hypersensitivity reactions to drugs are common in clinical practice. In some cases, the drugs responsible for such reactions are essential for the treatment of certain conditions and the only possibility is to perform desensitization to "induce drug tolerance". Usually this process takes several days.

Temozolomide is approved for the treatment of patients with malignant glioma (eg, glioblastoma multiforme or anaplastic astrocytoma) who experience recurrence or progression after standard therapy. It is also currently under clinical investigation for the treatment of brain metastases from a variety of cancers [1].

Temozolomide is an alkylating agent whose mechanism of action is to inhibit DNA replication. It is used as palliative therapy in gliomas and increases survival by several months.

It has been shown that the drug is active against human malignant gliomas when administered orally at a dosage of 200 mg/m^2 once daily for 5 days, in cycles of 28 days. Efficacy is assessed by radiology studies and neurologic status.

The most common side effects are nausea and vomiting. Severe myelosuppression, which manifests as thrombocytopenia and neutropenia, has also been reported.

Adverse reactions include urticaria [2], desquamative skin rash [3], anaphylaxis [4], pneumonitis [5], Stevens-Johnson syndrome and toxic epidermal necrolysis [6].

We describe 2 cases of delayed maculopapular rash that appeared after several days of treatment with temozolomide and recurred in the next cycle of treatment.

The first case was a 32-year-old woman diagnosed with neurofibromatosis type I, hamartoma in the left thalamus, and right temporal astrocytoma II-III who had been treated with surgery and radiotherapy in 1994 with complete remission.

In 2006 she was diagnosed with brain stem glioma. It was decided not to administer radiotherapy in view of the radiation doses she had received to date (up to 54 Gy). Chemotherapy was thus initiated with temozolomide.

In the fourth cycle she presented generalized pruritus and erythema, with maculopapular lesions. There were no other associated symptoms. The reaction started on day 3 to 4 of treatment and disappeared within 3 to 4 days with antihistamines and corticosteroids. During the next cycle, and again after 3 to 4 days of treatment, similar lesions appeared, leading to discontinuation of treatment.

Table. Desensitization Protocol With Temozolomide (Doses Administered Every 30 Minutes)

Dose, mg	Concentration	Volume Administered
0.035	0.025 mg/mL	1.4 mL
0.07	''	2.8 mL
0.15	''	6 mL
0.3	0.25 mg/mL	1.2 mL
0.75	''	3 mL
1.25	''	5 mL
2.5	''	10 mL
5	5 mg	1 tablet
10	''	2 tablets
20	20 mg	1 tablet
40	''	2 tablets
80	''	4 tablets
Remaining doses to reach the therapeutic dose		

In 2009, the patient's clinical condition deteriorated due to tumor progression, with spread to the middle cerebellar peduncle. Her oncologist decided to start treatment again with temozolomide. Considering the previous reactions to this drug, we designed a 1-day desensitization protocol (Table). After reaching the therapeutic dose of 280 mg, the patient received a single daily dose at home to complete 5 days of treatment.

The patient was able to complete 15 cycles of treatment with this protocol.

Patch tests were performed with temozolomide (5% in aqueous solution and 5% in petrolatum on the upper right arm) before starting the desensitization protocol. The results were negative when assessed at 48 and 96 hours.

The second case was a 65-year-old woman who had been diagnosed with glioblastoma multiforme in June 2009 and treated with surgery in January 2010. In April 2010, she began treatment with radiotherapy and chemotherapy with temozolomide (140 mg every 15 days). In June the dose was increased to 320 mg/d for 5 days every month.

Twelve days after the seventh cycle the patient developed generalized maculopapular rash. The lesions disappeared within 28 days with antihistamines and corticosteroids. There was no desquamation.

She was treated with systemic corticosteroids and therefore patch tests were not performed.

Temozolomide desensitization was performed following the same protocol described in case 1. The patient developed maculopapular lesions on her hands and wrists on the first day of the cycle, but these disappeared spontaneously in 24 hours.

In each new cycle the lesions appeared as before. They were mild, no premedication was administered, and the protocol was not changed. The patient received a total of 10 cycles and achieved clinical improvement when tumor progression stopped.

We have presented the results of successful desensitization in 2 patients with a delayed reaction to temozolomide and no therapeutic alternative.

It is widely accepted that it is important to distinguish between immediate and nonimmediate reactions [7] in order to adequately manage adverse drug reactions. Rapid desensitization is possible for immunoglobulin (Ig) E– and non-IgE–mediated reactions to drugs [8-10]. In delayed reactions, desensitization protocols are usually performed over several days.

To our knowledge, there are no reports of rapid desensitization protocols with chemotherapeutic agents in patients with delayed reactions in the literature. A recent study described successful desensitization with temozolomide in a woman who had experienced an anaphylactic reaction to this drug [4].

Neither of our patients had therapeutic alternatives, and both were able to complete their chemotherapy treatment thanks to the temozolomide desensitization protocol. When the drug is discontinued, tolerance is lost within hours or days, explaining why the procedure needs to be repeated before each new cycle.

In our cases, a rapid desensitization protocol proved safe and effective in patients with a delayed drug reaction. For this reason, we believe that rapid desensitization procedures can be considered in patients with delayed reactions.

Previous presentation: Case 1 was presented as an oral communication at the 2009 International Symposium on Drug Hypersensitivity organized by Spanish Society of Allergy and Clinical Immunology (SEAIC) in Logroño, Spain.

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Hev b 5: Latex Allergen Implicated in Clinically Relevant Cross-Reactivity With Manioc

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Key words: Allergen. Cross-reactivity. Food allergy. Latex. Manioc.

Palabras clave: Alérgeno. Reactividad cruzada. Alergia alimentaria. Látex. Yuca.

Manioc or cassava (*Manihot esculenta*) is a very important food in South America, Africa, and Asia. Native to Brazil, which is still one of the largest producers of this root vegetable, it was carried to Africa and Asia by Portuguese traders during the 16th century. The tuber, also known as cassava root, can be eaten boiled, fried, toasted or in the form of flour for bread, pastry, and cakes. It is the main source of carbohydrates for large populations in the tropics. Mainly eaten as a substitute for potato, manioc is slowly entering the European and North American diet, although it has been present for many years in the form of tapioca, which is dry starch obtained from cassava root.

In 2003 we published the first report of an allergic reaction to manioc [1]. In that paper, we proved by immunoglobulin (Ig) E immunoblotting inhibition analysis the existence of cross-reactivity between manioc and latex, leading to the inclusion of manioc on the growing list of foods involved in the latex-fruit syndrome. Afterwards, we described another latex-allergic patient with anaphylaxis to manioc [2], and 2 further cases were reported in Brazil [3]. Another case of anaphylaxis to manioc was reported in a Spanish woman in 2007 [4], and recently, 9 cases were reported in Brazil; they all had skin-related symptoms, such as urticaria and angioedema, and 3 patients had anaphylaxis to manioc [5].

The aim of the present study was to identify the latex allergen implicated in the latex-manioc cross-reactivity syndrome.

We included 2 patients with manioc-induced anaphylaxis, both of whom also had anaphylaxis to latex [1,2]. In both cases we performed skin prick tests (SPTs) with latex extract (ALK Abelló) and prick-to-prick tests with fresh manioc. Serum specific IgE to latex and manioc was measured by ImmunoCAP (Phadia), and the latex allergen sensitization pattern was studied using a panel of individual recombinant (rHev b 1, 3, 5, 6.01, 7, 8, 9, 10, 11 and 12) and native (nHev b 2 and nHev b 13) latex allergens, which were each coupled to ImmunoCAPs [6]. All the recombinant latex allergens were produced in *Escherichia coli* as a fusion protein with maltose-binding protein (MBP). MBP coupled on ImmunoCAPs served as a control. Sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblots were performed with manioc and latex extracts (AlaBLOT Specific IgE Procedure, DPC). Inhibition studies were performed by immunoblotting (AlaBLOT Inhibition Assay Procedure) and by ImmunoCAP inhibition.

Patient 1 was a 59-year-old Portuguese woman, born in Mozambique, with a previous history of asthma, severe latex allergy related to multiple surgeries, and latex-fruit syndrome. She had had an anaphylactic reaction with generalized urticaria, bronchospasm, and laryngeal edema 30 minutes after eating boiled manioc, and a similar reaction 5 minutes after eating raw manioc (tapioca flour). Previously, she had eaten manioc with no adverse reactions.

SPTs were positive to latex extract (9 × 7 mm) and fresh manioc (9 × 8 mm), and specific IgE was positive to latex (14.6

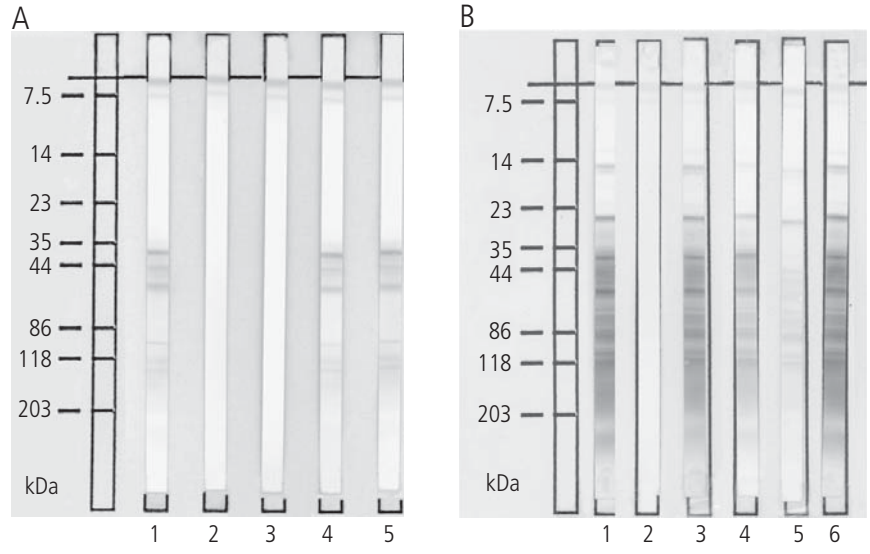

Figure. A. Manioc immunoblotting (AlaBLOT) immunoglobulin (Ig) E inhibition assay in patient 1. Lane 1, manioc immunoblotting (noninhibited); Lane 2, inhibited with manioc extract (10 µL of manioc extract, concentration of 10 mg protein/mL) as positive control; Lane 3, inhibited (100%) with latex allergen recombinant (r) Hev b 5 (concentration of 1.5 µg/µL); Lane 4, inhibited (5.7%) with latex allergen rHev b 7 (concentration of 3.2 µg/µL); Lane 5, inhibited with maltose-binding protein as negative control.
B. Manioc immunoblotting (AlaBLOT) IgE inhibition assay in patient 2. Lane 1, manioc immunoblotting (noninhibited); Lane 2, inhibited with manioc extract (as positive control); Lane 3, inhibited with latex allergen rHev b 7 (38.8%); Lane 4, manioc immunoblotting diluted 1:10 (noninhibited); Lane 5, inhibited with latex allergen rHev b 5 (64%); Lane 6, inhibited with maltose-binding protein as negative control.

kU/L) and manioc (5.1 kU/L). Specific IgE to individual latex allergens was positive only for rHev b 5 (19 kU/L) and nHev b 13 (0.81 kU/L). Immunoblotting of the manioc extract showed IgE-binding to 3 protein bands around 35, 42-44, and 50 kDa, which were 100% inhibited with latex allergen rHev b 5 (Figure 1A). In the ImmunoCAP inhibition study, we also observed 100% inhibition of manioc-specific IgE with rHev b 5 (concentration 1 μg/μL), and minor inhibition (24%) with nHev b 13 (concentration 0.75 μg/μL). In the cross-inhibition experiments, manioc extract (concentration 1 μg/μL) inhibited only 44% of the latex-specific IgE and 39% of the rHev b 5-specific IgE.

Patient 2 was a 46-year-old Portuguese woman, born in Guinea-Bissau, with a previous history of severe latex allergy related to occupational exposure (health care worker) and multiple surgeries, and latex-fruit syndrome. She had had an anaphylactic reaction with generalized pruritus, lip and hand angioedema, bronchospasm, and laryngeal edema 10 minutes after eating boiled manioc, and a similar reaction 5 minutes after eating raw manioc (tapioca flour). Previously, she had eaten manioc with no adverse reactions.

SPTs were positive to latex extract (5 × 5 mm) and fresh manioc (12 × 6 mm), and specific IgE was positive to latex (>100 kU/L) and manioc (40.1 kU/L). Specific IgE to latex allergens was positive to rHev b 1 (91.3 kU/L), nHev b 2 (1.5 kU/L), rHev b 3 (2.8 kU/L), rHev b 5 (>100 kU/L), rHev b 6.01 (>100 kU/L), rHev b 7 (0.5 kU/L), rHev b 8 (0.5 kU/L), rHev b 9 (0.6 kU/L), rHev b 10 (0.4 kU/L), rHev b 11 (31.9 kU/L), rHev b 12 (0.5 kU/L), and nHev b 13 (18.9 kU/L). Immunoblotting of the manioc extract showed IgE-binding to several protein bands, ranging from 16 to greater than 100 kDa, which were inhibited (64%) with latex allergen rHev b 5 (Figure 1B). In the ImmunoCAP inhibition study, we observed 100% inhibition of manioc-specific IgE with rHev b 5 and minor inhibition (ranging from 34%-49%) with nHev b 2, rHev b 7, rHev b 11, and nHev b 13 (concentration 0.16-1 μg/μL). In the cross-inhibition experiments, manioc extract inhibited only 15% of latex-specific IgE and only 26% of specific IgE to rHev b 5.

Our results strongly suggest that manioc allergy was a consequence of primary latex sensitization. The latex allergen responsible for this cross-reactivity was Hev b 5. This acidic structural protein is a strong antigen and one of the most important latex allergens, with a high prevalence in health care workers; furthermore, it has previously been associated with a homologous protein in kiwi fruit [7,8].

In accordance with recent findings suggesting that Hev b 5 may be a strong candidate for involvement in latex-manioc syndrome [5], and in contrast to a previous publication [9], we confirm for the first time, using IgE-binding inhibition studies, that Hev b 5 is the major latex allergen implicated in clinically relevant cross-reactivity with manioc. The immunoblotting inhibition results, where IgE-binding to several protein bands in the manioc extract was inhibited with rHev b 5, could be explained by the existence of multiple isoforms of Hev b 5 [8]. Another explanation could be the presence of multiple IgE-binding epitopes, since 11 epitopes have been identified to date, making Hev b 5 a multivalent allergen [10].

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Neuropsychiatric Reactions to Montelukast

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Key words: Montelukast. Asthma. Suicide. Psychic symptoms. Headache.

Palabras clave: Montelukast. Asma. Suicidio. Síntomas psíquicos. Cefalea.

Montelukast is a very useful therapeutic tool for persistent asthma. Clinical practice guidelines such as the Spanish Guidelines for Asthma Management (GEMA) and the Global Initiative for Asthma (GINA) describe leukotriene inhibitors as an important step for the treatment and management of mild to moderate persistent asthma. Their utility lies in the inhibition of proinflammatory substances, leukotrienes, released from various cells. The leukotrienes bind to CysLT receptors found in the human airway and cause increased mucus secretion, enhanced vascular permeability, bronchoconstriction, and eosinophilia [1].

Although montelukast is considered a safe drug [2-4], there have been reports of several adverse reactions since it was first marketed in 1998. These include hypersensitivity reactions, reactions involving the hepatic, cardiovascular, and digestive systems, and neuropsychiatric effects. These last effects are very difficult to diagnose when the symptoms cannot be linked to the prescribed treatment.

In our experience, the most common effect is headache, although children aged between 1 and 5 years old are frequently affected by sleep disorders, such as insomnia, somnolence, and night terrors, as well as behavioral disturbances and mood disorders. There have also been reports of anorexia, extreme aggressiveness, hallucinations, paresthesia/hypoesthesia, and autolytic ideation.

In 2007, the Spanish pharmacovigilance system reported 70 cases of psychiatric disorders, such as those previously described, in patients treated with montelukast [5].

In 2009, a group of researchers evaluated the relationship between montelukast and suicidal acts in approximately 20 000 adults and children receiving this treatment. The results showed 1 case in which a suicide attempt could be linked to montelukast treatment. The study concluded that this relationship was unusual because there were no significant differences between the active and the control groups [6].

In 2011 the US Food and Drug Administration linked 838 cases of attempted suicide to antileukotriene treatment, but montelukast was involved in just 5 cases [7].

We report on 4 children who presented neuropsychiatric side effects and mood disorders while receiving treatment with montelukast for asthma control. None of them had previous known psychiatric disorders. The clinical features are shown in the Table.

All the children presented extreme aggressive behavior. In fact, patient 3 was admitted to the child psychiatry unit because of aggressive behavior and autolytic ideation. At the time he was taking 10 mg daily of montelukast and salbutamol as rescue medication. In 2006, he had experienced a manic crisis attributed to prolonged treatment with inhaled corticosteroids, although he had also been taking montelukast 10 mg continuously for at least 2 years. Good tolerance to inhaled corticosteroids was confirmed and once montelukast was withdrawn all the psychiatric symptoms disappeared.

In our experience, time to onset of symptoms from exposure to the drug ranges from 4 days to 3 months. Once montelukast was withdrawn all of the patients improved within 24 hours or several days at the most.

Although the adverse effects observed in our patients were attributed to the use of montelukast, there are also data

Table. Clinical Characteristics of Children Who Presented Neuropsychiatric Reactions to Montelukast

Patient	Sex	Age, y	Personal History (PH) and Family History (FH) of Behavioral Disturbances	Asthma Treatment	Exposure Time to Montelukast	Neuropsychiatric Symptoms	Time to Resolution
1	Male	9	PH: none FH: none	Budesonide salbutamol, and montelukast 5 mg	3 wk	Day 4: headache and behavioral disturbances. Day 20: headache and behavioral disturbances, aggressiveness, and night terrors	24-48 h
2	Male	14	PH: none FH: none	Salbutamol and montelukast 5 mg	3 mo	Extreme aggressiveness	5 d
3	Male	14	PH: manic crisis in 2006 attributed to corticosteroids FH: schizophrenia	Salbutamol and montelukast 10 mg	3 wk	Behavioral disturbances, extreme aggressiveness, and autolytic ideation	10 d
4	Male	8	PH: none FH: none	Salbutamol, specific immunotherapy, and montelukast 5 mg	6 wk	Extreme aggressiveness, behavioral disturbances, and night terrors	7 d

suggesting that these effects might actually be due to the allergy itself [8,9].

It is interesting that all our patients were male because in most of the published studies, these psychiatric disorders in children are more common in females [5].

Despite the above, montelukast is still regarded as a safe, well-tolerated drug which is very useful in the long-term treatment of asthma. Nevertheless, in our opinion, a careful medical history enquiring about behavioral disturbances should be carried out before starting treatment with montelukast. It is important to be familiar with the risk of neuropsychiatric reactions in order to identify and stop them.

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Hypereosinophilic Syndrome Associated With Regulatory T-Cell Disruption as a Complication of Stem Cell Transplantation

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Key words: Hypereosinophilic syndrome. Regulatory T cell. Multiple myeloma. Stem cell transplant. $CD25^+$ regulatory T cell.

Palabras clave: Síndrome hipereosinofílico. Célula T reguladora. Mieloma múltiple. Célula T reguladora $CD25^+$.

Hypereosinophilic syndromes (HES) are disorders that manifest with marked peripheral blood and tissue eosinophilia associated with organ dysfunction. HES may result from a clonal myeloid stem cell disorder or a clonal proliferation of T cells with aberrant cytokine production [1]. Nonetheless, many cases have an undefined cause and the etiology of the eosinophilic infiltration is poorly understood. Furthermore, clinical factors predisposing to the development of HES are unknown.

Regulatory T cells (Tregs) are lymphocytes with a primary role in the maintenance of immune system homeostasis. A subgroup of these cells express high levels of the interleukin (IL) 2 receptor alpha chain (CD25) and low levels of the IL-7 receptor alpha chain (CD127) on their cell surface. These $CD25^{bright}CD127^{dim}$ Tregs are potent modulators of cytokine responses which can affect eosinophil function [2].

Herein, we describe a case of HES following autologous stem cell transplantation for multiple myeloma with an associated expansion of the $CD25^{bright}CD127^{dim}$ Treg compartment and augmented Treg suppressive function. We propose that the immune dysregulation underlying at least some forms of HES may be secondary to disruption of the $CD25^{bright}CD127^{dim}$ Treg compartment.

A 58-year-old female received autologous stem cell transplantation for multiple myeloma. Three weeks following transplant, the patient developed whole body erythroderma with exfoliation and peripheral eosinophilia up to 18000 cells/μL (reference <500 cells/μL). Two months later, she developed pneumonitis and hepatitis. Biopsies demonstrated eosinophilic infiltrates in the lung, liver, and skin. The time to symptom development was inconsistent with engraftment syndrome, and autologous graft-vs-host disease was unlikely based on biopsies of multiple organs showing no evidence of this.

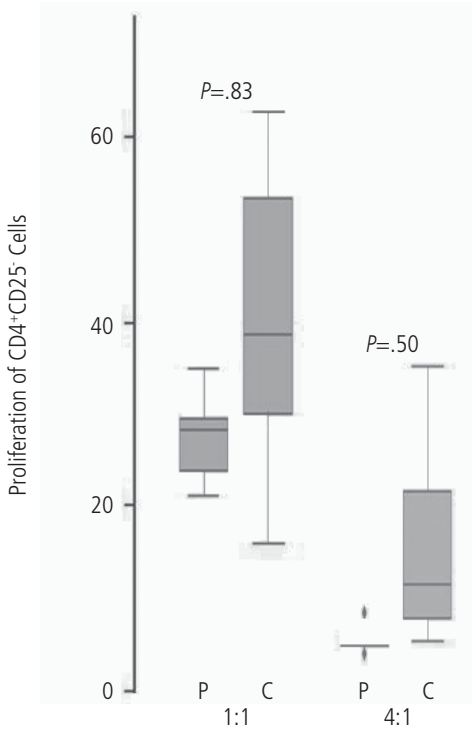

Figure. $CD4^+CD25^{bright}$ cell-mediated inhibition of $CD4^+CD25^-$ cell proliferation. Results are presented as relative inhibition of proliferation of 20 000 $CD4^+CD25^-$ cells at 1:1 and 4:1 ratios with $CD4^+CD25^{bright}$ cells as compared to proliferation of $CD4^+CD25^-$ cells alone [9]. *P* indicates patient; C, controls.

Drug reaction and infection were eliminated as causes by discontinuing medications and obtaining appropriate cultures. A 2-week course of prednisone led to improvement, but upon discontinuation, the erythroderma returned.

Laboratory evaluation revealed hypogammaglobulinemia with a peripheral B-cell count of 0 cells/μL (reference 70-910 cells/μL). $CD3^+$ T cell count was 4900 cells/μL (reference 710-4180 cells/μL), with 3700 cells/μL being $CD4^+$ and 1200 cells/cells/μL being $CD8^+$. There was no evidence of a clonal T-cell population by flow cytometry or T-cell receptor gene rearrangement PCR. Cytogenetic testing for myeloid neoplasm, including *PDGFRA/FIP1L1* fluorescent in situ hybridization, was negative. Based on the persistent peripheral eosinophila, end-organ eosinophilic infiltration, and absence of clonal cells, the patient was diagnosed with idiopathic HES.

The patient was started on immunomodulatory doses of intravenous immunoglobulin at 1 g/kg every 4 weeks for both immunoglobulin replacement and antieosinophilic effects [3,4]. The patient refused steroid therapy and her dermatitis worsened. Six months later intravenous mepolizumab (anti-IL5) therapy was initiated at 750 mg every 4 weeks. Peripheral eosinophil count decreased from 6740 to 60 cells/μL over 2 months. The erythroderma improved moderately but was a continued problem; therefore, cyclosporine was started at 200 mg/d with the eventual addition of prednisone 20 mg/d to obtain full control. Both oral medications were weaned off over 20 months. The patient continues on mepolizumab with no symptoms, end-organ infiltration, or eosinophilia.

Absolute lymphocyte counts (mean [SD] number of cells/μL) were similar between the patient (1645 [120] and 10 healthy controls (2211 [496]), *P*=.153, but the percentage of lymphocytes that were $CD3^+CD4^+$ was marginally lower in the patient (36.0% [7.1]) compared to the controls (47.7% [7.7]), *P*=.076.

The number of $CD25^{bright}CD127^{dim}$ Tregs was greater in the patient compared to the controls when expressed as an absolute number of cells (251 [12] vs 59 [22], *P*<.001) or as a percent of $CD4^+$ cells (43.3% [7.4%] vs 5.8% [2.0%], *P*<.001). $CD4^+CD25^{bright}$ cells from the patient were more effective at suppressing the proliferation of $CD4^+CD25^-$ cells in comparison to 4 healthy controls (Figure). On a cell-to-cell basis, $CD4^+CD25^{bright}$ cells from the patient were 2.8 times more suppressive of $CD4^+CD25^-$ cell proliferation than those from controls (*P*=.050).

Serum IL-5, the primary regulator of eosinopoiesis and eosinophil activation, was undetectable at onset of symptoms, but after initiation of mepolizumab therapy increased to 7.0 pg/mL. Commercially available assays measure free IL-5 and not IL-5 bound to mepolizumab (GlaxoSmithKline, personal communication). Other pro-eosinophilic cytokines also had elevated serum levels, with higher levels during disease exacerbation than during periods of disease control (tumor necrosis factor-α: 131.0 vs 6.0 pg/mL; IL-13: 12.0 vs <10.6 pg/mL).

We describe expansion and augmented function of the $CD25^{bright}CD127^{dim}$ Treg compartment as a possible cause of a nonclonal lymphocytic variant of HES. The presence of an expanded population of functionally augmented $CD25^{bright}CD127^{dim}$ Tregs associated with cytokine perturbations promoting eosinophilic inflammation suggests that these cells are involved in the development of the cytokine imbalance leading to HES. Finally, the immune perturbations experienced during stem cell transplantation may facilitate the development of this disorder as seen in the described patient.

Increased numbers of $CD25^{bright}CD127^{dim}$ Tregs have been implicated in disorders with prominent components of eosinophilic inflammation such as eosinophilic esophagitis [5]. Furthermore, Tregs have been shown to have a reduced ability to suppress the secretion of pro-eosinophilic type 2 helper T cell (T_H2) cytokines in comparison to T_H1 cytokines [6]. These findings support the role of $CD25^{bright}CD127^{dim}$ Tregs in the pathogenesis of HES in the presented patient as the highly elevated numbers of $CD25^{bright}CD127^{dim}$ Tregs may preferentially suppress T_H1 cytokines in relation to T_H2 cytokines, resulting in uncontrolled eosinophilic inflammation.

Disruption of the Treg compartment as the cause of HES in this patient is supported by the elevated serum levels of pro-eosinophilic lymphokines and clinical responsiveness to inhibition of 1 of these cytokines (IL-5). The disruption is unlikely to be secondary to her malignancy because symptoms were not present prior to transplantation. Moreover, Tregs are present in low numbers and have compromised function in multiple myeloma [7]. Furthermore, the expansion of the Treg compartment seen in the presented patient is unlikely to be due to direct effects of stem cell transplantation because Treg numbers do not reach supra-normal levels after immune reconstitution [8].

The presented data support the hypothesis that $CD25^{bright}CD127^{dim}$ Treg dysfunction may lead to some forms of the lymphocytic variant of HES, and suggest that HES should be considered when eosinophilic inflammation is present after hematopoietic stem cell transplantation.

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Strongyloides stercoralis Infection: A Series of Cases Diagnosed in an Allergy Department in Spain

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Palabras clave: *Strongyloides stercoralis*. Eosinofilia. Asma. Urticaria.

Strongyloidiasis is caused by *Strongyloides stercoralis*, an intestinal nematode usually acquired by walking barefoot on infested soil. *S stercoralis* infects hundreds of millions of people, mostly in tropical and subtropical areas. In Spain a prevalence of 12.4% has been detected on the Mediterranean coast and there have been isolated reports of disseminated strongyloidiasis [1]. In recent years, there has been an increase in cases of imported helminthiasis in Spain because of immigration from endemic areas and international travel.

In contrast to other helminthic parasites, *S stercoralis* can complete its life cycle entirely within the human host, allowing for replication of the parasite and life-long persistence [2]. Chronic infections with *S stercoralis* can be clinically inapparent or lead to cutaneous (larva currens, pruritus, urticaria), gastrointestinal (abdominal bloating or discomfort, diarrhea, nausea, and anorexia) or pulmonary symptoms (cough and shortness of breath). In most cases there is associated eosinophilia [1,2].

In patients with depressed cell-mediated immunity, autoinfection may give rise to potentially fatal hyperinfection with disseminated disease [3,4].

Definitive diagnosis is usually based on the detection of larvae in stool specimens but because of the low output of larvae, a standard stool examination is very insensitive (<50 %). Repeated stool examinations and the agar plate culture method are highly efficient for the detection of *S stercoralis* infection [5]. Serodiagnosis with enzyme-linked immunosorbent assay, which detects serum immunoglobulin (Ig) G against a crude extract of infective larvae is highly sensitive and specific [6].

We present 9 patients with eosinophilia (>450 cells μL) and a serologic diagnosis of strongyloidiasis and report their outcomes after treatment.

An initial detailed clinical history and physical examination were conducted. Skin prick tests with common aeroallergens, laboratory tests including complete blood count with differential, total and specific IgE, chest radiography, and spirometry were performed in patients consulting for asthma. In patients with chronic urticaria we ordered complete blood count with differential, liver and renal function tests, erythrocyte sedimentation rate, C3 and C4 assays, determination of thyroid-stimulating hormone levels, and

Table. Demografic and Clinical Characteristics, Serology Results, and Eosinophilia Values Before and After Antihelmintic Treatment

Patient	Sex/ Age, y	Presenting Complaint	Additional Symptoms	Positive Skin Prick Test	Pretreatment Eosinophilia, Cells/μL (%)	Posttreatment Eosinophilia, Cells/μL (%)	Serology Results for Other Parasites, kU/L
1	F/ 44	Seasonal rhinitis and asthma		Pollens and dust mites	1280 23.9%	190 4.5%	IgG *Toxocara canis*: positive IgE *Anisakis simplex*: 2.08 IgE *Ascaris lumbricoides*: 3.83 IgE *Equinoccocus*: ND
2	M/40	Seasonal rhinitis and asthma Chronic urticaria		Pollens and dander	1270 15.7%	480 6.2%	IgG *Toxocara canis*: positive IgE *Anisakis simplex*: 0.46 IgE *Ascaris lumbricoides*: 0.78 IgE *Equinoccocus*: 0.03
3	F/34	Eosinophilia Abdominal pain Cutaneous pruritus		None	1170 13.4%	420 5.8%	IgG *Toxocara canis*: positive IgE *Anisakis simplex*: 0.85 IgE *Ascaris lumbricoide*s: 1.15 IgE *Equinoccocu*s: 0.04
4	F/39	Seasonal rhinitis and asthma Chronic urticaria		Pollens	930 14.9%	440 6.4%	IgG *Toxocara canis*: ND IgE *Anisakis simplex*: 0.65 IgE *Ascaris lumbricoides*: 0.52 IgE *Equinoccocus*: 0.09
5	F/40	Seasonal rhinitis and asthma	Nonspecific abdominal complaints	Pollens	1630 21%	501 8.1%	IgG *Toxocara canis*: negative IgE *Anisakis simplex*: 1.28 IgE *Ascaris*: ND IgE *Equinoccocus*: 0.56
6	F/61	Eosinophilia Severe asthma	Chronic urticaria Nonspecific abdominal complaints	None	1480 20%	140 1.8%	IgG *Toxocara canis*: ND IgE *Anisakis simplex*: 0.18 IgE *Ascaris lumbricoides*: 0.06 IgE *Equinoccocus*: 0.03
7	F/35	Seasonal rhinitis and asthma	Nonspecific abdominal complaints	Pollens and dander	1400 14%	530 6.5%	IgG *Toxocara canis*: positive IgE *Anisakis simplex*: 0.40 IgE *Ascaris lumbricoides*: 1.06 IgE *Equinoccocus*: ND
8	M/38	Chronic urticaria/angioedema	Occasional episodes of dyspnea	None	1500 20.5%	243 3%	IgG *Toxocara canis*: ND IgE *Anisakis simplex*: 3.0 IgE *Ascaris lumbricoides*: 6.52 IgE *Equinoccocus*: 0.43
9	F/41	Persistent rhinitis and asthma	Past acute urticaria	Pollens	2580 33.6%	430 9.8%	IgG *Toxocara canis*: positive IgE *Anisakis simplex*: 0.80 IgE *Ascaris:* ND IgE *Equinoccocus:* ND

Abbreviations: Ig, immunoglobulin; ND, not done.

autoantibodies to thyroglobulin and thyroid peroxidase, a skin prick test to *Anisakis simplex*, and stool examination. When eosinophilia was detected, a stool examination and serologic test for parasites (specific IgE against *A simplex*, *Ascaris lumbricoides*, and *Echinococcus granulosus*, and specific IgG against *Toxocara canis* and *S stercoralis*) were ordered.

Patients with positive serology for parasites were treated with ivermectin (200 mcg/kg in 2 single doses) and/or albendazole (400 mg twice daily for 5 days) [7]. A follow-up visit with blood tests was conducted.

The study included 9 adults (7 women) with an age of between 35 and 61 years. All but 1, a Spanish woman diagnosed with intrinsic asthma and chronic eosinophilic pneumonia (patient 6), were from Latin America. The corresponding demographic and clinical characteristics, serologic test results, and eosinophilia values before and after antihelmintic treatment are shown in the Table.

Standard examination of stool specimens was negative in all patients but they all had a positive serologic test for *S stercoralis*. All of them had eosinophilia (range 930-2580 cells/μL) and total serum IgE ranged from 148 to 2280 UI/mL. The results of the other serologic tests for parasitic infections are shown in the Table. None of the patients had a current or past clinical history of allergy to *A simplex* or anisakiasis.

Eosinophilia decreased after treatment in all patients, although the changes in total serum IgE were irregular. Clinically, there was an improvement in patients with digestive and cutaneous symptoms.

Currently, screening for strongyloidiasis is appropriate for individuals with clinical manifestations (including unexplained eosinophilia) and epidemiologic exposure, immunosuppressed patients with unexplained eosinophilia, and asymptomatic individuals who have been in areas known to be endemic [2,8].

Most of our patients consulted for seasonal rhinitis and asthma but the detection of elevated eosinophilia and the fact that they were from endemic areas led us to test for parasitic infections. The diagnosis was made by serologic testing. One disadvantage of this method is that it can detect cross-reactivity with other helminths, including *A lumbricoides* and *A simplex* [2,6,8]. In addition, patients with strongyloidiasis frequently harbor other parasites [8].

While it is important to correctly diagnose and treat all parasite infections, this is critical in strongyloidosis as complications may arise, including the potentially lethal hyperinfestation syndrome. Conditions that may require corticosteroid treatment (eg, asthma), are a risk factor. Even short courses of corticosteroids of 6 to 17 days have led to overwhelming hyperinfection and death [3,4,9].

Treatment with ivermectin or albendazole is usually well tolerated. Eosinophilia persisting for several months after treatment suggests either failure to eradicate *S stercoralis* and/or other causes of eosinophilia [8]. In our patients, eosinophilia decreased in all cases after treatment, suggesting that the parasite was successfully eradicated.

Previous presentation: Some of these results were presented as a poster at the XXVII National Congress of the Spanish Society of Allergy and Clinical Immunology (SEAIC) in Madrid, Spain (2010).

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Sublingual Immunotherapy: Factors Influencing Adherence

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Allergen immunotherapy administered sublingually (SLIT) is considered an effective option for the treatment of respiratory allergy [1]. The sublingual route involves the daily administration of allergen doses for 2 to 3 years and adequate adherence is crucial to the success of treatment. Adherence to SLIT has been analyzed by several studies [2,3]. A follow-up schedule consisting of 3-monthly visits may significantly improve adherence [4], but a study analyzing the opinion of allergists reported that effectiveness, cost, and tolerability rather than regular follow-up were major factors influencing adequate SLIT adherence [5].

In order to assess the factors that might exert an influence on adherence to SLIT in a real-life setting, we analyzed 241 patients with allergic rhinitis (perennial or seasonal), with or without asthma, treated with SLIT. Treatment was performed according to the manufacturer's instructions and the patients were monitored in 3 visits: at baseline and at 6 and 12 months. Potential factors influencing adherence were analyzed by means of 2 questionnaires at each visit: 1 for the physician and 1 for the patient. Fifty percent of the patients were aged 14 years or younger while 42% were aged 18 years or older; 74% were from public hospitals and the remaining 26% were from private offices. Rhinitis was seasonal in 42% of the group and perennial in 58%; 63% had associated asthma. The most frequent allergens involved were mites (49%), grass (38%), trees (28%), *Alternaria alternata* (9.5%), and cat and dog epithelia (5%).

Before starting treatment, 78.3% of patients declared that they were well informed about the characteristics of their allergic disease and 68.7% accepted the limitations caused by the disease, even though 76% considered that it worsened their quality of life. This last outcome was statistically different between children and adults (69.9% vs 82.1%, *P*=.0317). The difference remained significant for those with perennial allergy (children: 69.9%, adults: 90.9%, *P*=.0039) but not for those with seasonal allergy.

Six months after initiating treatment, there were no statistical differences in the number of patients who continued with treatment, independently of age or type of allergic disease (seasonal or perennial). However, after 1 year of treatment, statistical differences were found between children and adults (91.4% vs 73.3%, *P*=.0008) and between seasonal and perennial disease (68.0% vs 92.1%, *P*<.001). The difference between children and adults was also significant in the case of seasonal rhinitis (81.8% vs 57.8%, *P*=.0246) but not in that of perennial disease. The proportion of patients with perennial disease who attended the follow-up visits was 97.1% in the case of children and 78.6% in that of adults (*P*=.0017)

Most of the patients considered SLIT to be a convenient treatment and did not view tolerability as a cause for concern. When asked if they occasionally forgot to take the treatment, statistically significant differences were found between those with seasonal disease and those with perennial disease (52.6% vs 36.3%, *P*=.0260) after 6 months. There were no significant differences according to age.

In our study, the factors with the greatest influence on SLIT adherence after 1 year of treatment were age and type of disease (perennial or seasonal). With 3 visits in 1 year (baseline and 2 follow-up visits), the percentage of adherence in children (91.4%) was almost identical to that reported by de Vita et al [4] (91.9%) for a group of patients with 4 visits in 1 year. The age of patients in both studies was similar. Röder et al [2], in a randomized trial, found that the factors that influenced adherence were age, difficulty in following medication instructions, and overall evaluation of treatment. In our study, SLIT was considered a convenient treatment and overall evaluation of treatment had no influence on adherence. Age was particularly relevant in those with seasonal disease but not in those with perennial disease, probably because parental influence on children is greater in the case of the former.

In conclusion, the factors with the greatest impact on adherence to SLIT in a real-life setting were age (children vs adults) and type of allergic disease (perennial vs seasonal). Other factors such as convenience of treatment or severity of allergic disease were not found to be relevant.

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