Toll-like Receptor-9 Gene Polymorphism in Common Variable Immunodeficiency

S Tanir,[1] M Karkucak,[2] T Yakut[2], SS Kilic[1]
[1]*Department of Pediatrics, Division of Pediatric Immunology, Uludag University Medical Faculty, Bursa, Turkey*
[2]*Department of Medical Genetics, Uludag University Medical Faculty, Bursa, Turkey*

Key words: Common variable immunodeficiency. Toll-like receptor 9. Polymorphism.

Palabras clave: Inmunodeficiencia común variable. Receptor tipo Toll 9. Polimorfismo.

Common variable immunodeficiency (CVID) is a primary immunodeficiency involving a heterogeneous group of disorders characterized by recurrent bacterial infections and deficient production of different classes of antibodies. The predisposing genetic factors associated with CVID remain unknown [1], although homozygous defects in *ICOS* or *CD19* have been detected in a very small number of patients [2,3].

Toll-like receptors (TLRs), one of the tools of the innate immune system, are involved in initiating signaling pathways of immune and inflammatory genes. TLR9, which recognizes unmethylated CpG-DNA sequences, is expressed at high levels in B cells. TLR9 signaling via CpG-DNA sequences plays a direct role in initiating and sustaining humoral immunity as well as memory B cell responses [4,5]. Recent studies have revealed broad TLR9 defects in CVID consisting of both a lack of TLR9 expression and defective TLR9 function in B cells [6].

Twenty single nucleotide polymorphisms (SNPs) have been identified in CVID. To evaluate the candidacy of TLR9 as a CVID susceptibility gene in the Turkish population, we investigated the association between the TLR9 -1237T/C promoter polymorphism and CVID.

Our study included 30 CVID patients and 29 age-matched healthy controls. The study was approved by the medical ethics committee of Uludag University and informed consent was obtained from all patients and/or their parents or guardians.

Genomic DNA was obtained from peripheral blood leukocytes. We genotyped the TLR9-1237T/C promoter polymorphism using polymerase chain reaction-restriction fragment length polymorphism analysis. Genomic DNAs were amplified by 5′-CCTGCTTGCAGTTGACTGTG-3′(forward primer) and 5′-CCCTGTTGAGAGGGTGACAT-3′ (reverse primer), followed by BstNI restriction enzyme (5 U/μL; Genemark) digestion [7].

There were no significant differences between patient and control groups in terms of age or sex. Analysis of TLR9 gene polymorphisms showed that 9 CVID patients (30%) and 6 healthy controls (20%) had the TC genotype. The TT genotype (wild-type) was detected in 21 CVID patients and 23 controls. None of the individuals included in the study displayed the CC genotype (Figure).

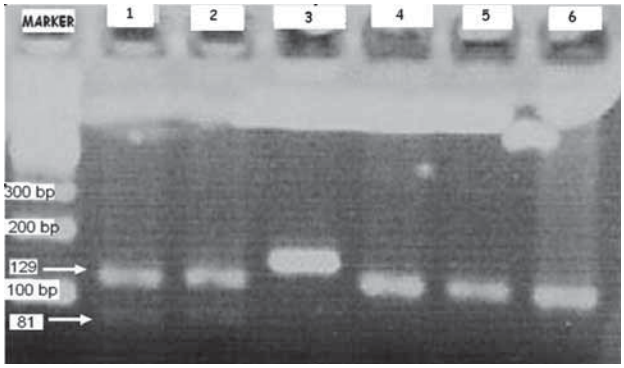

Figure. Detection of TLR9 -1237T/C promotor polymorphism by polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis. Lanes 1 and 2 are from the samples of the T/C genotype (129 base pairs [bp], 81 bp, 48 bp and 25 bp). Lane 3 is from uncut PCR product(154 bp). Lanes 4 to 6 are from samples from the T/T genotype (129 bp and 25 bp).

TLR defects have been reported in CVID. Branda et al [8] showed that B cells in patients with CVID had an impaired ability to proliferate or secrete immunoglobulins when stimulated with oligonucleotides containing CpG DNA. They also found that the lack of B-cell activation in response to CpG DNA was independent of the B-cell memory phenotype. Since late B-cell differentiation is abnormal in CVID, Cuningham et al [6] investigated the effects of CpG DNA on B cells in patients with CVID. Upon activation with CpG DNA, TLR9 appeared on the surface of memory B cells of healthy controls, but was undetectable or barely detectable in the patients. Their study also showed that the B cells of CVID patients had markedly reduced amounts of TLR9 protein and TLR9 mRNA. None of the TLR9 SNPs were present in CVID patients.

In the present study we have analyzed the TLR9 SNP -1237 T/C in a group of patients with CVID. A comparison of patients and controls revealed no differences in terms of TT (wild-type) or TC genotypes. In order to highlight the potential candidacy of the TLR9 gene as a susceptibility marker for CVID, further studies with other TLR9 SNPs are required.

References

1. Cunningham-Rundles C. Common variable immunodeficiency. Curr Allergy Asthma Rep. 2001;1:421-9.
2. Grimbacher B, Hutloff A, Schlesier M, Glocker E, Warnatz K, Dräger R, Eibel H, Fischer B, Schäffer AA, Mages HW, Kroczek RA, Peter HH. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. Nat Immunol. 2003 ;4:261-8.

3. van Zelm MC, Reisli I, van der Burg M, Castaño D, van Noesel CJ, van Tol MJ, Woellner C, Grimbacher B, Patiño PJ, van Dongen JJ, Franco JL. An antibody-deficiency syndrome due to mutations in the CD19 gene. N Engl J Med. 2006 4;354:1874-7.
4. Krieg AM, Yi AK, Matson S, Waldschmidt TJ, Bishop GA, Teasdale R, Koretzky GA, Klinman DM. CpG motifs in bacterial DNA trigger direct B-cell activation. Nature 1995. 6: 374: 546-9.
5. Bernasconi NL, Onai N, Lanzavecchia A. A role for Toll-like receptors in acquired immunity: up-regulation of TLR9 by BCR triggering in naive B cells and constitutive expression in memory B cells. Blood. 2003. 1;101:4500-4.
6. Cunningham-Rundles C., Radigan L,. Knight AK, Zhang L, Bauer L, Nakazawa A. TLR9 Activation Is Defective in Common Variable Immune deficiency. J Immunol. 2006 .176:1978-87.
7. Hong J, Leung E, Fraser AG, Merriman TR, Vishnu P, Krissansen GW. TLR2, TLR4 and TLR 9 polymorphisms and Crohn's disease in a New Zealand Caucasian cohort. J Gastroenterol Hepatol. 2007.22:1760-6.
8. Branda RF, Moore A L, Hong R, McCormack JJ, Zon G, Cunningham-Rundles C. B-cell proliferation and differentiation in common variable immunodeficiency patients produced by an antisense oligomer to the rev gene of HIV-1. Clin Immunol Immunopathol. 1996.79:115-21.

Manuscript received August 31, 2001; accepted for publication, November 26, 2009.

Sara Sebnem Kilic
Department of Pediatric Immunology
Uludag University Medical Faculty
Bursa,Turkey
E-mail: sebnemkl@uludag.edu.tr

Late Asthmatic Reaction Induced by Exposure to Raw Swiss Chard

T Valbuena[1], P Barranco,[2] C Pascual,[1] MC López-Serrano,[2] S Quirce[2]
[1]*Allergy Unit, Hospital Infanta Sofía, San Sebastián de los Reyes, Madrid, Spain*
[2]*Allergy Unit, Hospital La Paz, Madrid, Spain*

Key words: Asthma. Swiss chard. Vegetables. Specific inhalation challenge.

Palabras clave: Asma. Acelga. Verduras. Provocación inhalatoria específica.

Swiss chard (*Beta vulgaris* L. cicla group), a vegetable belonging to the Chenopodiaceae family, is widely consumed in Spain. Allergic symptoms caused by ingestion of foods are common, but in some instances they may also occur due to the inhalation of volatile food antigens [1,2].

We report the case of a 52-year-old housewife with a history of allergic rhinoconjunctivitis due to grass pollen who experienced 2 episodes of wheezy dyspnea and cough within 8 to 10 hours of handling raw Swiss chard at home. She had never previously experienced symptoms following the inhalation of vapor from Swiss chard or spinach, or contact with or ingestion of these vegetables.

Skin prick tests were performed with a series of common airborne allergens, food allergens (Swiss chard, spinach), and purified allergens such as profilin and peach lipid transfer protein (ALK-Abelló, Madrid, Spain). Prick by prick tests with raw Swiss chard, spinach, and sugar beet were also performed. Positive results were obtained for *Lolium, Olea, Cupressus,* and *Chenopodium* pollen, raw Swiss chard, and spinach. A skin patch test with raw Swiss chard was negative.

Total serum immunoglobulin (Ig) E and specific IgE (Phadia ImmunoCAP, Uppsala, Sweden) to spinach, sugar beet, rBet v 1, and profilin (rBet v 2) were determined. Total serum IgE was 803 kU/L. Specific IgE (kU/L) was positive for sugar beet (2.62) and rBet v 1 (2.16).

At baseline, the methacholine challenge test was negative, serum eosinophilic cationic protein (ECP) was 15.8 mcg/L, and the fraction of exhaled nitric oxide (Fe_{NO}) was 50.8 parts per billion (ppb). A specific inhalation challenge (SIC) simulating real-life conditions was performed by asking the patient to handle Swiss chard as she would do at home [3-7]. The test was carried out in an 18-m^3 room where the patient cleaned and cut the vegetable for 10 minutes. Forced vital capacity, forced expiratory volume in 1 second (FEV_1) and peak expiratory flow (PEF) were measured at baseline, every 10 minutes for the first hour after the SIC, and then every hour for 4 hours. Thereafter, PEF was measured hourly for 24 hours (not counting sleep time). The SIC elicited a 20% fall in PEF from baseline with dyspnea 10 hours after the test, and a 32% fall in FEV_1 24 hours after the challenge. No immediate asthmatic reaction was observed as measured by serial FEV_1 and PEF readings (Figure). Twenty-four hours after the SIC, the methacholine challenge was positive (20% decrease in FEV_1 from baseline caused by 2.4 mg/mL) and Fe_{NO} levels had increased significantly (91.7 ppb).

As a control challenge test (sham exposure) the patient handled lettuce for 10 minutes and no asthmatic reaction was observed. Another patient with allergic asthma due to grass pollen did not react to a SIC with Swiss chard.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis and IgE-immunoblotting with raw and cooked Swiss chard were carried out. Several IgE-binding bands were observed in

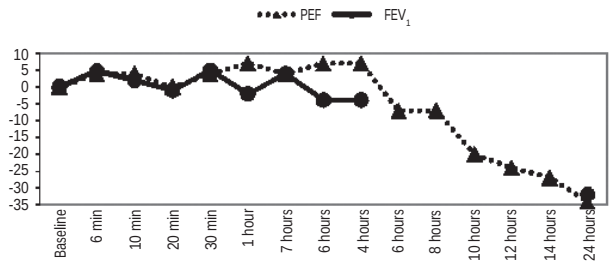

Figure. Serial peak expiratory flow (PEF) and forced expiratory volume in 1 second (FEV_1 readings) during and 24 hours after the challenge.

the raw extract with the patient's serum (molecular masses of 16, 33, 36, 45, 70, 80, and 92 kDa), but no bands were observed with the cooked Swiss chard extract.

This is an unusual case of asthma due to raw Swiss chard handling in a patient who tolerated cooking vapors and subsequent ingestion of this vegetable. The SIC test elicited an isolated late asthmatic reaction, and an increase in airway hyperresponsiveness to methacholine and Fe_{NO} was observed 24 hours after the challenge test. The fact that the patient tolerated cooked Swiss chard and its vapors during cooking shows that the allergen responsible for her respiratory symptoms was thermolabile and can be destroyed by cooking and/or digestion. To the best of our knowledge, this is the first report of a patient with asthma due to inhalation of vegetables who developed an isolated late asthmatic reaction.

Acknowledgments

These data were presented in poster form at the XXVI Congress of the Spanish Allergology and Clinical Immunology Society (SEAIC) in Bilbao, Spain 2008.

References

1. James JM, Crespo JF. Allergic reactions to foods by inhalation. Curr Allergy Asthma Rep. 2007;7:167-74.
2. Quirce S, Madero MF, Fernández-Nieto M, Jiménez A, Sastre J. Occupational asthma due to the inhalation of cauliflower and cabbage vapors. Allergy. 2005;60:969-70.
3. de la Hoz B, Fernandez-Rivas M, Quirce S, Cuevas M, Fraj J, Dávila I, Igea JM, Losada E. Swiss chard hypersensitivity: clinical and immunologic study. Ann Allergy. 1991;67:487-92.
4. González-Mancebo E, Alfaya T, Pulido Z, León F, Cuevas M, de la Hoz B. Swiss chard-induced asthma. Allergy. 2000;55:511-2.
5. Parra FM, Lázaro M, Cuevas M, Ferrando MC, Martin JA, Lezaun A, Alonso MD, Sanchez-Cano M. Bronchial asthma caused by two unrelated vegetables. Ann Allergy. 1993;70:324-7.
6. Daroca P, Crespo JF, Reaño M, James JM, Lopez-Rubio A, Rodriguez J. Asthma and rhinitis induced by exposure to raw green beans and chards. Ann Allergy Asthma Immunol. 2000;85:215-8.
7. Igea JM, Fernandez M, Quirce S, de la Hoz B, Diez Gomez ML. Green bean hypersensitivity: an occupational allergy in a homemaker. J Allergy Clin Immunol. 1994;94:33-5.

Manuscript received September 7, 2009; accepted for publication, December 14, 2009.

Teresa Valbuena
Unidad de Alergia, Hospital Infanta Sofía
Paseo de Europa 34, 28702
San Sebastián de los Reyes
Madrid, Spain
E-mail: teresa17d@hotmail.com

Allergic Sensitization to Ethylene Oxide in Patients With Suspected Allergic Reactions During Surgery and Anesthesia

MS Opstrup, H Mosbech, LH Garvey
Danish Anaesthesia Allergy Centre, Allergy Unit, Copenhagen University Hospital, Gentofte Hospital, Denmark.

Key words: Ethylene oxide. Anaphylaxis. Specific IgE. Hypersensitivity. Anesthesia.

Palabras clave: Óxido de etileno. Anafilaxia. IgE específica. Hipersensibilidad. Anestesia.

Ethylene oxide (ETO) is a highly reactive gas, widely used for sterilizing heat-sensitive medical devices [1] such as catheters, infusion sets, gloves, suture sets, surgical drapes, and certain implantable materials such as ventriculoperitoneal (VP) shunts [2]. The toxicity of ETO is well-established [3], but ETO can also cause immunoglobulin (Ig) E– mediated allergic sensitization. Myelomeningocele and hemodialysis patients appear to be high-risk groups, with a reported prevalence of sensitization in these groups of 23% [4] and 12.1% [5], respectively. Although all patients are exposed to ETO during surgery and anesthesia, no studies have investigated the incidence of allergic sensitization in such patients. The objective of this study was to investigate the frequency of allergic sensitization to ETO in patients with suspected allergic reactions during anesthesia.

All patients investigated at the Danish Anaesthesia Allergy Centre from May 2004 to June 2009 (n=201) were included in the study. They had symptoms ranging from mild skin symptoms to severe anaphylactic shock. Specific IgE to ETO was measured in stored serum (ImmunoCAP, Phadia AB, Uppsala, Sweden). All blood samples had been drawn at least 4 to 6 weeks after the suspected allergic reaction. For patients with detectable IgE to ETO, additional serum samples from the time of the suspected reaction and a later sample were analyzed to follow the development in IgE over time.

Three (1.5%) of 201 patients had elevated specific IgE to ETO (>0.35 kU_A/L); the remaining tests were negative. Changes in specific IgE to ETO over time are shown in the Figure. All 3 patients had clinical anaphylaxis with severe hypotension and skin symptoms, and all had another verified allergy (cefuroxime, hydroxyethyl starch, and latex). However, only 2 of the patients (those sensitized to cefuroxime and hydroxyethyl starch) were exposed to this allergen prior to the reaction. Two of the 3 patients belonged to already identified high-risk groups. The first (also sensitive to latex) had myelomeningocele and the second (also sensitive to cefuroxime) was a hemodialysis patient.

Our study shows that specific IgE to ETO can vary over time. In a study of dialysis patients in whom exposure to ETO was avoided over an 8-week period, specific IgE to ETO fell in all the patients and became undetectable in 50% of these [6]. Re-exposure to ETO for 4 weeks produced renewed elevation

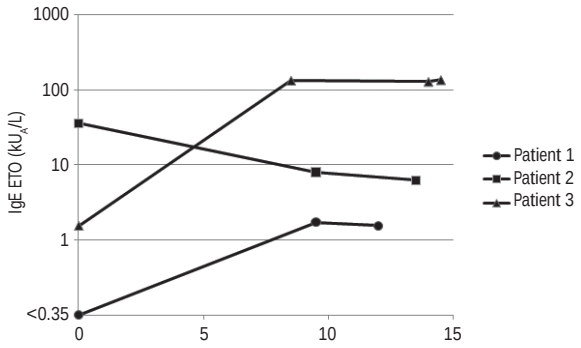

Figure. Changes in specific immunoglobulin E (IgE) to ethylene oxide (ETO) over time.

of IgE in all the patients and clinical symptoms recurred or worsened in some. This relationship with time and exposure has also been reported for IgE to chlorhexidine [7]. Also, a recent report on IgE-mediated allergy to chlorhexidine suggested that clinically relevant sensitization could still be present at levels of between 0.35 kU_A/L and 0.20 kU_A/L, ie, below the detection limit of commercially available kits, especially if exposure to the allergen has been avoided for a long period [8]. The fact that ETO is highly toxic, a hapten, and a gas makes it difficult to produce standardized material for skin tests, challenge studies, and in vitro tests such as the histamine release test. Nonetheless, such additional assays would be valuable to support diagnosis. In conclusion, sensitization to ETO in patients with allergic reactions during anesthesia is rare (1.5%). The clinical relevance of sensitization to ETO remains to be confirmed since the 3 patients who had elevated specific IgE to ETO in our study had another verified allergy. However, it is likely that sensitization to ETO, either alone or as an enhancing factor, is capable of causing an anaphylactic reaction [9,10], especially in the context of the high-level exposure to ETO during surgery and anesthesia.

Due to the widespread use of ETO in the health service, we recommend that testing for sensitization to ETO should be included in investigations of patients with suspected allergic reactions in connection with surgery and anesthesia.

Acknowledgments

The reagents used in the analysis of specific IgE to ETO was provided free of charge by Phadia AB.

References

1. Demontoux V, Moreau E, Peyron I, Talon V, Khouri N, Morin JP, Bézie Y. Surgery in a patient allergic to ethylene oxide. Pharm World Sci. 2008;30:208-9.
2. Pittman T, Williams D, Rathore M, Knutsen AP, Mueller KR. The role of ethylene oxide allergy in sterile shunt malfunctions. Br J Neurosurg. 1994;8:41-5.
3. Thier R, Bolt HM. Carcinogenicity and genotoxicity of ethylene oxide: new aspects and recent advances. Crit Rev Toxicol. 2000;30:595-608.
4. Pittman T, Kiburz J, Steinhardt G, Krock J, Gabriel K. Ethylene oxide allergy in children with spina bifida. J Allergy Clin Immunol. 1995;96:486-8.
5. Marshall C, Shimizu A, Smith EK, Dolovich J. Ethylene oxide allergy in a dialysis center: prevalence in hemodialysis and peritoneal dialysis populations. Clin Nephrol. 1984;21:346-9.
6. Bommer J, Ritz E. Ethylene oxide (ETO) as a major cause of anaphylactoid reactions in dialysis (a review). Artif Organs. 1987;11:111-7.
7. Garvey LH, Kroigaard M, Poulsen LK, Skov PS, Mosbech H, Venemalm L, Degerbeck F, Husum B. IgE-mediated allergy to chlorhexidine. J Allergy Clin Immunol. 2007;120:409-15.
8. Nagendran V, Wicking J, Ekbote A, Onyekwe T, Garvey LH. IgE-mediated chlorhexidine allergy: a new occupational hazard? Occup Med. 2009;59:270-2.
9. Poothullil J, Shimizu A, Day RP, Dolovich J. Anaphylaxis from the product(s) of ethylene oxide gas. Ann Intern Med. 1975;82:58-60.
10. Lemke HD, Heidland A, Schaefer RM. Hypersensitivity reactions during haemodialysis: role of complement fragments and ethylene oxide antibodies. Nephrol Dial Transplant. 1990;5:264-9.

Manuscript received November 11, 2009; accepted for publication January 21, 2010.

Morten Schjørring Opstrup
Danish Anaesthesia Allergy Centre
Allergy Unit K 816, Department of Dermato-Allergology
Gentofte Hospital
Niels Andersens Vej 65
DK-2900 Hellerup, Denmark
E-mail: mortenop@hotmail.com

Pattern of Sensitization to Major Allergens Der p 1 and Der p 2 in Mite-Sensitized Individuals from Galicia, Spain

V Iraola,[1] M Boquete,[2] H Pinto,[1] F Carballada,[2] C Carballás,[2] J Carnés[1]
[1]Research & Development Department, Laboratorios LETI S.L., Madrid, Spain
[2]Allergy Service, Hospital Xeral de Calde, Lugo, Spain

Key words: *Dermatophagoides pteronyssinus*. Allergens. Der p 1. Der p 2. Specific IgE.

Palabras clave: *Dermatophagoides pteronyssinus*. Alérgenos. Der p 1. Der p 2. IgE específica.

Der p 1 and Der p 2 are the main sensitizing allergens of *Dermatophagoides pteronyssinus* and the most significant contributors to total immunoglobulin (Ig) E reactivity [1,2]. It has been also documented that the IgE-binding frequency of these allergens varies considerably depending on the

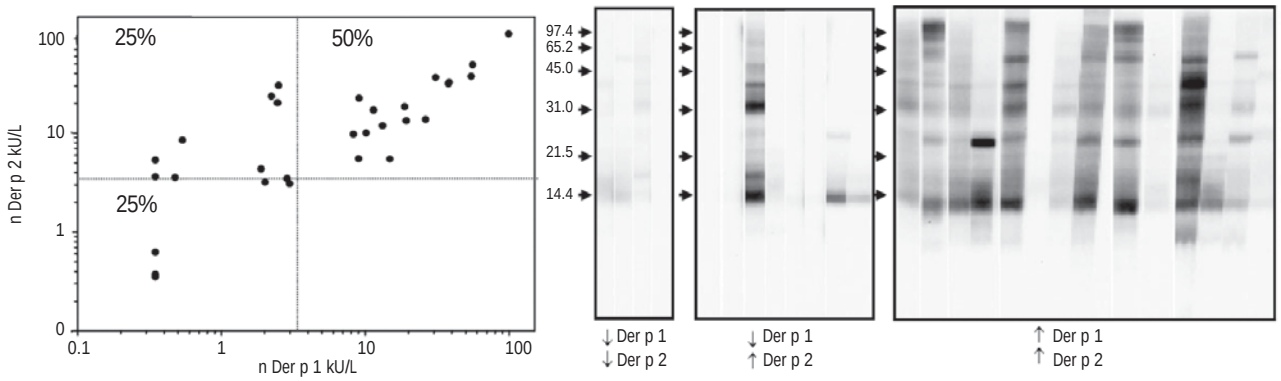

Figure. Distribution of Der p 1– and Der p 2–specific immunoglobulin E levels in patient sera. The dash line indicates the threshold line of 3.50 kU/L (class 3). Negative values are represented as 0.35 kU/L. Immunoblot analysis results are shown by subgroups.

geographical origin of the population [3,4] where individuals can be sensitized to one or both allergens.

We describe the distribution of specific IgE to Der p 1 and Der p 2 in 32 allergic individuals (37.6% females), with a mean (SD) age of 28.2 (15.6) years, residing in Lugo (Galicia, Spain). The individuals analyzed had asthma (15.6%), rhinitis (50%), or both (34.4%), were monosensitized to mites, and had a positive skin prick test (SPT) to *D pteronyssinus* extract (Laboratorios LETI, Madrid, Spain) containing 12 µg/mL and 8 µg/mL of Der p 1 and Der p 2, respectively. Wheal size was measured by papulometry. Serum samples were taken after informed oral consent was obtained. Specific IgE (sIgE) to *D pteronyssinus* (Phadia, Sweden) and natural purified Der p 1 and Der p 2 (Indoor Biotechnologies, Chalottesville, Virginia, USA) were measured by UniCAP (Phadia, Uppsala, Sweden). sIgE levels were subdivided into 2 groups: *a)* low levels (<3.50 kU/L) and *b)* medium/high levels (≥3.50 kU/L). Allergenic profile was determined by immunoblot analysis. Results were analyzed using contingency tables (Fisher exact test) and nonparametric tests (Kruskal-Wallis, Tukey, Spearman correlation).

Mean (SD) sIgE to the whole *D pteronyssinus* extract was 37.2 (33.9) kU/L; 81.3% of individuals showed sIgE to Der p 1 (15 [16.3] kU/L) and 90.6% to Der p 2 (16.3 [20.1] kU/L); 93.7% were sensitized to Der p 1 and/or Der p 2, 78.1% had detectable levels to both allergens, 12.5% were sensitized to Der p 2 only, 3.1% were sensitized to Der p 1 only, and 6.3% tested negative to both allergens. There was a significant difference between sIgE to *D pteronyssinus* and to Der p 1 ($P<.05$). This difference was not significant for Der p 2. Significant correlations were obtained between sIgE to *D pteronyssinus* and to both Der p 1 (0.864, $P<.001$) and Der p 2 (0.883; $P<.001$). There was no significant correlation between IgE levels and wheal sizes. Twenty-five percent of the individuals showed high levels of sIgE to Der p 2 and no or low levels to Der p 1 ($P=.002$) (Figure). Immunoblot analysis was performed with sera from 27 patients. There was an evident difference in patterns of recognition between subgroups, with an increased number of bands recognized in serum samples from the low Der p 1/high Der p 2 subgroup and the high Der p 1/high Der p 2 subgroup, in particular, compared to the low Der p 1/low Der p 2 subgroup. No significant differences were observed between the groups for symptoms or wheal sizes.

Our results confirm previous published findings that indicate that over 95% of patients have detectable IgE to Der p 1 and/or Der p 2 [1,3]. Our percentage of sensitization to Der p 1 (81%) is lower than prevalence rates observed in other European countries (85%-100%). However, the corresponding percentage for Der p 2 (91%) is similar to figures reported for Central Europe [4]. A similar profile of sensitization, with predominant sensitization to Der p 2, has been observed in Austrian patients [1]. The discrepancies observed in our study–undetectable sIgE in patients with a positive SPT and a lack of correlation between sIgE to mite allergens and SPT results—have been previously reported [5,6]. Differences in the allergenic profile of sensitization and clinical implications of high sensitization to Der p 2 but not to Der p 1 remain to be elucidated.

Since the use of recombinant allergens is being proposed for a more accurate diagnosis of mite-allergic patients [4], the sensitization profile of different populations is important for a correct diagnosis. According to our results we can conclude that approximately 94% of mite-sensitized individuals can be diagnosed correctly with the inclusion of Der p 1 and Der p 2 in the diagnostic test.

References

1. Pittner G, Vrtala S, Thomas WR, Weghofer M, Kundi M, Horak F, Kraft D, Valenta R. Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. Clin Exp Allergy. 2004;34(4):597-603.
2. Hales BJ, Martin AC, Pearce LJ, Laing IA, Hayden CM, Goldblatt J, Le Souëf PN, Thomas WR. IgE and IgG anti-house dust mite specificities in allergic disease. J Allergy Clin Immunol. 2006;118(2):361-7.

3. Boquete M, Iraola V, Fernández-Caldas E, Arenas Villaroel L, Carballada FJ, González de la Cuesta C, López-Rico MR, Núñez Orjales R, Parra GA, Soto-Mera MT, Varela S, Vidal C. House dust mite species and allergen levels in Galicia, Spain: a cross-sectional, multicenter, comparative study. J Investig Allergol Clin Immunol. 2006;16(3):169-76
4. Weghofer M, Thomas WR, Kronqvist M, Mari A, Purohit A, Pauli G, Horak F, Grönlund H, van Hage M, Valenta R, Vrtala S. Variability of IgE reactivity profiles among European mite allergic patients. Eur J Clin Invest. 2008 Dec;38(12):959-65
5. van der Zee JS, de Groot H, van Swieten P, Jansen HM, Aalberse RC. Discrepancies between the skin test and IgE antibody assays: study of histamine release, complement activation in vitro, and occurrence of allergen-specific IgG. J Allergy Clin Immunol. 1988;82(2):270-81
6. Taketomi EA, Silva DA, Sopelete MC, Gervásio AM, Alves R, Sung SJ. Differential IgE reactivity to Der p 1 and Der p 2 allergens of Dermatophagoides pteronyssinus in mite-sensitized patients. J Investig Allergol Clin Immunol. 2006;16(2):104-9.

Manuscript received September 28, 2009; accepted for publication, January 26, 2010.

Jerónimo Carnés
Research & Development
Laboratorios LETI, S.L.
sCalle del Sol nº 5
28760 Tres Cantos
Madrid, Spain
E-mail: jcarnes@leti.com

