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

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Keywords: Common variable immunodeficiency. Toll-like receptor 9. Polymorphism.

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’-CCTGTTGCACTTGACGTG-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 promoter 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

Late Asthmatic Reaction Induced by Exposure to Raw Swiss Chard

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


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-Abello, 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 (FeNO) 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-m² room where the patient cleaned and cut the vegetable for 10 minutes. Forced vital capacity, forced expiratory volume in 1 second (FEV1) 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 FEV1 24 hours after the challenge. No immediate asthmatic reaction was observed as measured by serial FEV1 and PEF readings (Figure). Twenty-four hours after the SIC, the methacholine challenge was positive (20% decrease in FEV1 from baseline caused by 2.4 mg/mL) and FeNO 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
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 FeNO 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


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

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


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 3 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/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
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 0.35 kU/L and 0.20 kU/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.

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


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

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


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
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 p teronyssinus 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 p teronyssinus (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). Allergic 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 p teronyssinus 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 p teronyssinus and to Der p 1 (P<.05). This difference was not significant for Der p 2. Significant correlations were obtained between sIgE to D p teronyssinus 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


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.

