Usefulness of Specific-IgG4 to Hymenoptera Venom in the Natural History of Hymenoptera Stings

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Key words: Hymenoptera stings. Hornet venom. Specific IgG4. Specific IgE.

Palabras clave: Picadura de himenóptero. Veneno de avispón. IgG4 específica. IgE.

Hymenoptera venom hypersensitivity can be effectively detected by measuring serum venom-specific (s)IgE [1]. Normal immunity to allergens is characterized by predominant IgE and/or IgG formation, especially in the IgG4 class [2]. In patients allergic to aeroallergens, the finding of serum IgG4 antibodies directed against allergens to which the patients are not sensitive suggests that these antibodies are not sensitizing [3]. Furthermore, sIgG4 is thought to block sIgE to venom, and is elevated by venom immunotherapy [4]. However, a potential role for sIgG4 in individuals with venom sensitivity to natural stings has not yet been clearly established. We examined sIgG4 response to Hymenoptera venom and the utility of measuring sIgG4 and sIgE in individuals who had experienced Hymenoptera stings

The Table shows the characteristics of the 274 study participants and 20 controls. Severity of systemic reactions (SRs) was graded according to Mueller [5] as follows: Grade 0, 168 participants; Grade 1-3 (mild), 84 participants; and Grade 4 (severe), 22 participants, The total number of stings occurring in a period of no longer than 5 years was classified as few (1-5 stings) in 96 participants and many (≥6 stings) in 178. All the participants completed questionnaires and underwent peripheral blood tests. The study was approved by the Dokkyo Medical University Research Ethics Committee and written informed consent was obtained from each participant prior to study enrolment.

There were irregular intervals between the last sting and the time of peripheral blood collection. sIgE and sIgG4 to hornet venom were measured by Mitsubishi Chemical Co., SRL Co., and Phadia Co. in Japan, respectively. Detection of sIgE with the CAP system using fluorescent ELISA can be expressed in quantitative units (kU/L) or using the traditional spectrum of 7 semi-quantitative classes, ranging from class 0 (<0.35 kU/L) to class 6 (>100 kU/L). In this study, sIgE-positive results (mean [SD] 4.47 [8.82] kU/L) were defined as levels above <0.35 kU/L (≥class 1).

Japanese forestry workers are frequently exposed to hornet stings. For this reason, serum hornet venom sIgG4 was

Table. Characteristics of 274 Participants With a Natural History of Hymenoptera Stings and 20 Controls

	No. of Individuals	M/F (No.)	Age (Mean/ Range), y	No. of Systemic Reactions[a]	sIgE (Mean [SD])	sIgG4/sIgE[b] (No. [%] of Individuals)	No. of Previous Stings	sIgG4 (+/-) (No. of Individuals)	sIgG4 (mean [SD], ng/mL
Controls	20	18/2	32.6/25-51	ND	<0.35 kU/ml	-/- 20 (100)	0	0/20	<0.07
Study Participants	274	264/10	49.6/19-79	0: 168 1-3 (mild): 84 4 (severe): 22	4.47 (8.82)	-/- 22 (8) -/+ 52 (19) +/- 22 (8) +/+178 (65)	Few (1-5): 96	58/38	0.42 (1.03)[c]
							Many (≥6):178)	142/36	1.19 (1.74)[c,d]
							Total: 274	200/74	1.10 (2.17)

Abbreviations: F, female; M, male; ND, not done; sIgE, specific IgE, sIgG4, specific IgG4.

[a]Graded according to severity using the Mueller classification [5].

[b]The Fisher exact test was used to assess the significance of correlation between sIgG4 and sIgE levels. - and + indicate a negative and positive result, respectively. A positive test for sIgG4 and sIgE to hornet venom was defined by a level of ≥0.07 ng/mL and ≥0.35kU/mL, respectively.

[c]$P<.001$ as compared with levels of sIgG4 in individuals who have never been stung.

[d]$P<.001$ as compared with levels of sIgG4 in individuals with few stings.

measured by ELISA. The assay used was able to detect sIgG4 concentrations of 0.07 ng/mL and above.

Data are presented as means (SD). The Mann-Whitney U test was used to assess the significance of differences between groups and the Fisher exact test to assess the significance of correlation between sIgG4 and sIgE results. *P* values of less than .05 were considered to indicate statistical significance. Statistical analyses were performed with JMP software (Version 7.0 for MAC, SAS Institute).

We investigated the relationship between sIgG4 levels (mean [SD],1.10 [2.17] ng/mL) and the number of stings in the 274 individuals studied. sIgG4 levels and sIgE results in the 20 participants (controls) who had never experienced Hymenoptera stings were <0.07 ng/mL and class 0, respectively. Levels of sIgG4 were significantly higher in persons with few stings and many stings than in those who had never been stung (P<.001in both cases). They were also significantly higher in individuals with many stings than in those with few stings (P<.001). Finally, sIgG4 levels were not significantly affected by the presence of a mild or severe SR compared with no SRs.

There was no significant correlation between sIgG4 and sIgE values and we therefore analyzed the presence of positive and negative results. sIgG4 and sIgE positivity was observed in 178 participants (65.0%), while sIgG4 and sIgE negativity was observed in 22 participants (8.0%). sIgG4 negativity and sIgE positivity occurred in 52 participants (19.0%), while sIgG4 positivity and sIgE negativity occurred in 22 (8.0%). There was a significant correlation between sIgG4 and sIgE (P=.002).

sIgE levels do not correlate with the severity of SRs and some patients with barely detectable sIgE can have near-fatal anaphylaxis [6]. sIgE is also found in patients without a history of reactions or with local reactions only. Several studies have shown undetectable sIgE in individuals with clear SRs to stings [2]. sIgE is negative in about 18% of patients with SRs, whereas the intradermal skin test is negative in only about 2% [7]. Taken together, sIgE results should be evaluated carefully with consideration of the patient's clinical course, as false negatives may be influenced by other factors such as venom exposure duration.

sIgG4 appears to induce a change in sIgE production [8]. This induction may be one of the allergic mechanisms involved [8]and may also indicate the approximate number of past stings and the likelihood of SRs in the future. We analyzed the potential role of sIgG4 in relation to sIgE in individuals with venom sensitivity. The level of sIgG4 increased with frequency of stings. These findings are consistent with a mechanism demonstrated in other research in which IL-4 and IL-10 production from type 2 helper T cells and regulatory T cells, induced by immunological mechanisms, led to a switch from sIgE to sIgG4 [9]. However, levels of sIgG4 in individuals in our group were lower than those induced by venom immunotherapy and natural desensitization in beekeepers [2] and might not be high enough to block sIgE. Nonetheless, sIgG4 in our participants may have incompletely reduced the risk of SRs to subsequent stings. Furthermore, in our study sIgG4 positivity was observed in 50% of persons with negative sIgE, suggesting that sIgG4 and IgE may serve as markers of past stings and the likelihood of future allergic reactions. On the other hand, sIgG4 negativity and sIgE positivity are thought to be present immediately after stings or to indicate a false-positive sIgE result, with involvement of cross-reactive carbohydrate determinants [10]. sIgG4-positive and sIgE-negative tests may reflect the high sensitivity of the testing tool or indicate that a long time has elapsed since the last sting. sIgG4 positivity could indicate a diagnosis of allergy in individuals with negative sIgE, while negative results for both sIgG4 and sIgE are thought to indicate an absence of an allergic constitution.

In conclusion, we examined sIgG4 response to Hymenoptera venom in previously stung individuals. Our results indicate that measurement of sIgG4 might be useful in the diagnosis of an allergic constitution and as a marker of previous stings.

Acknowledgments

We thank Mrs. Reiko Komura and Mrs. Hiroko Kawazu for their skillful technical assistance.

Funding

This study was supported by a Health and Labour Sciences Research Grant (H21-Roudou- Wakate-007), Japan.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Manuscript received April 20, 2013; accepted for publication, June 16, 2013.

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Occupational Asthma Caused by Exposure to *Ceratitis capitata* (Mediterranean Fruit Fly)

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Key words: *Ceratitis capitata* allergy. Diptera. Fly allergies. Occupational asthma. Specific inhalation challenge test.

Palabras clave: Alergia por *Ceratitis capitata*. Alergia por dípteros. Asma ocupacional. Test de provocación inhalatoria específica.

The Mediterranean fruit fly, *Ceratitis capitata* (medfly), is a species of true fly (order Diptera, suborder Brachycera) in the family Tephritidae. It is one of the most crop-damaging pests in the Mediterranean. Fruit fly pest species have been successfully controlled and managed using the sterile insect technique, a control strategy that involves the infertile mating of sterile males (Vienna-8 strain) with wild females to reduce pest populations [1]. Several cases of occupational allergy due to different species of Diptera have been reported [2-6]. The first case of possible occupational asthma involving IgE-mediated hypersensitivity caused by exposure to medfly was described in 2010 in a worker at a company producing sterile male flies; however, specific bronchial challenge (SBC) to medfly was not carried out [7]. Herein, we report 2 new cases of occupational asthma in workers from the same company.

The first patient was a 29-year-old woman who had worked for 4 years at the company where Vienna-8 strain medflies are produced. In the year prior to consultation, she had developed rhinoconjunctivitis, which had worsened in the previous 4 months, with episodes of paroxysmal cough and dyspnea with wheezing, predominantly at night. Symptoms would appear in connection with exposure to the working environment (mainly in the room where the adult flies were housed), persist for some hours at home, and resolve over the weekends and during vacations. Skin tests with common air allergens were performed, yielding positive results for grass pollen (3 mm), without clinical relevance. A skin prick test with medfly extract (Bial-Aristegui) was positive (3.5 mm), as was serum specific IgE (14 kU/L, EAST, Bial-Aristegui; results >0.35 kU/L are considered positive). Pulmonary function testing showed normal baseline spirometry and a baseline fraction of exhaled nitric oxide (Fe_{NO}) of 32 ppb. A methacholine challenge test was positive; the concentration of methacholine required to cause a 20% fall in forced expiratory volume in the first second (PC_{20}) was 0.395 mg/mL. Serial measurements of peak expiratory flow (PEF) were performed in the patient's working environment and after her shift ended, and showed significant reductions (exceeding 20%) in PEF values, especially at the end of the shift and at home during

the night. SBC to medfly was carried out using a previously described method [8] with some modifications. The dose was increased in tenfold increments at 10-minute intervals. PC_{20} allergen was calculated using logarithmic interpolation by formula with the last 2 concentrations [9]. SBC to medfly extract elicited an early asthmatic response (PC_{20}, 0.24 mg/mL). No late asthmatic responses were observed. A 106% increase in Fe_{NO} was observed 24 hours after the test (66 ppb vs 32 ppb at baseline). We performed SDS-PAGE as described by Laemmli [10] and immunoblotting with medfly body extract, which revealed IgE binding bands at approximately 80 kDa, 66 kDa, 60 kDa, 33 kDa, and 27.5 kDa (Figure); these bands were similar to molecular masses described by Peláez et al [7] in research carried out with serum from a worker at the same biological facility.

The second patient was a 43-year-old man who had worked for 6 years at another biological facility of the same company where the sterile fly pupae are raised. Onset of clinical manifestations had occurred over the previous 6 months; these included nasal discharge and coughing fits that progressively worsened, followed by nasal congestion, sneezing, and wheezing in connection with exposure to the working environment during cleaning of ventilation ducts or exposure to rooms with a higher density of flies. The symptoms reappeared at night, but resolved during weekends and vacations. Skin tests for common air allergens yielded negative results. A skin prick test with medfly extract was positive (6.5 mm), as was serum specific IgE (14.4 kU/L). Pulmonary function tests revealed normal baseline spirometric values and a baseline Fe_{NO} of 14 ppb. The SBC to medfly extract elicited an early asthmatic response (PC_{20}, 0.04 mg/mL). No late asthmatic responses were observed. An 85% increase in Fe_{NO} was observed 24 hours after the test (26 ppb vs 14 ppb at baseline). SDS-PAGE-immunoblotting assay with adult medfly extract revealed IgE binding bands at 16 kDa and 13 kDa (Figure). An immunoblotting assay using adult medfly extract and an anti-prawn tropomyosin serum from rabbit was carried out; the detected bands showed different molecular masses to those revealed with the sera from both patients.

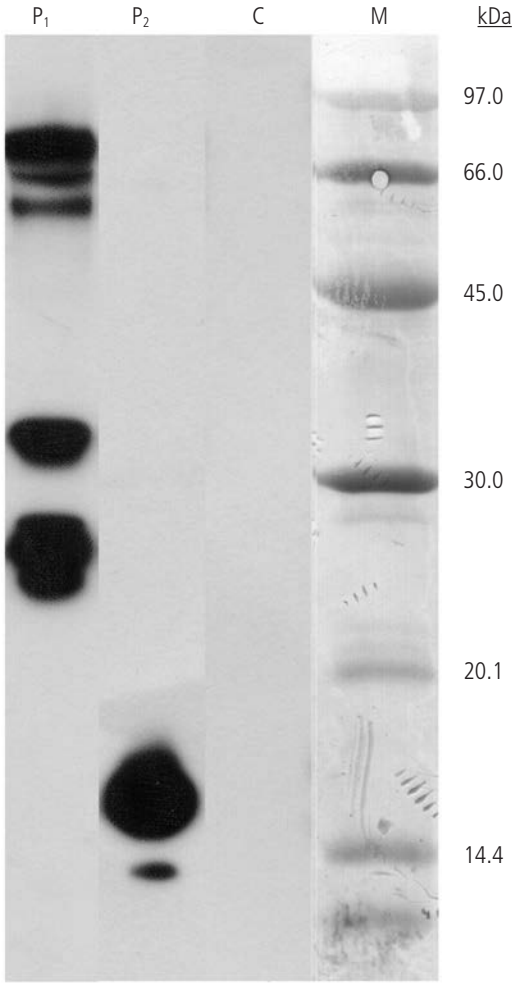

Figure. SDS-PAGE immunoblotting results. *Ceratitis capitata* extract. Lane P1, serum from patient 1; Lane P_2, serum from patient 2; Lane C, control serum (pool of sera from nonatopic individuals); Lane M, molecular mass marker.

Both patients provided written informed consent to the performance of bronchial challenge tests. Skin tests with medfly extracts were negative in 3 healthy controls and 2 asymptomatic exposed workers (1 of whom was atopic).

We have presented 2 cases of allergy by inhalation of proteins derived from the body of the Mediterranean fruit fly, *C capitata*, both involving respiratory symptoms indicative of rhinitis and asthma. The etiological implication of exposure to this fly as an occupational allergen has been demonstrated for the first time by means of a positive SBC. Differences in patterns of exposure to the fly, related to the activities carried out by each of the patients, may explain the different IgE-binding profiles revealed in *C capitata* extract with both sera. Further studies are required to elucidate these aspects and their potential clinical relevance.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Manuscript received May 6, 2013; accepted for publication, June 11, 2013.

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Recurrent Anaphylaxis Associated With Solitary Bee Sting (Hymenoptera: Megachilidae) in a Patient With Mastocytosis

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Key words: Hymenoptera. Sting. Megachile. Mastocytosis. Solitary bee.

Palabras clave: Alergia a himenópteros. Veneno. Megachile. Mastocitosis. Abeja solitaria.

Mastocytosis is an uncommon disease resulting from a monoclonal proliferation of pathological mast cells in different tissues including skin, bone marrow, liver, spleen, lymph nodes, and the gastrointestinal tract [1]. Insect stings are the main cause of anaphylaxis in mastocytosis [2]. It is estimated that 30% of all mastocytosis patients experience venom anaphylaxis, and these reactions are often more severe than in the general insect venom–allergic population [3]. Because of the dramatic increase in severe sting-related anaphylaxis in patients with mastocytosis, physicians should consider clonal mast cell disease in anyone with unexplained anaphylaxis or sting-related anaphylaxis [4].

The insects responsible for the most severe allergic stings reactions are hymenoptera of the Apidae, Vespidae, and Formicidae families. The Apidae family includes honey bees and bumble bees, while the Vespidae family includes the subfamilies Vespinae (*Vespula, Dolichovespula*, and *Vespa*) and Polistinae (*Polistes* species) [5], among which *Polistes dominula* (European paper wasp) is widespread, especially in Mediterranean areas [6].

We present the case of a 43 year-old man working as a bricklayer in a rural area with a clinical history of anaphylaxis by sensitization to wasp venom (specific IgE to *P dominula*, 1.45 kU/L). The patient also had osteoporosis. He was under treatment with allergen specific immunotherapy to *P dominula* venom, which was well tolerated. After a year of immunotherapy, he developed a cutaneous eruption that was diagnosed as purpura pigmentosa chronica, and in addition, he reported 4 episodes of anaphylaxis after an insect sting during work. The patient supplied some specimens of the insect for identification and future testing.

The insect was identified as a leaf-cutter bee of the genus *Megachile* (Hymenoptera; Apoidea; Megachilidae), specifically *Megachile* (*Eutricharaea*) *concinna* (Figure 1), the pale leafcutting bee, which is a solitary bee with a Holarctic distribution. A skin prick test using an extract of *M concinna* bodies (1:20 w/v) was performed, with negative results.

Hematological and immunological tests showed normal total IgE levels (26.1 kU/L), low specific IgE levels to *P dominula* (0.5 kU/L), and high levels of basal serum tryptase

Figure. *Megachile concinna* (Hymenoptera: Megachilidae).

(21.9 mg/L). The other results of the complete blood count and biochemical blood tests were normal, except for slightly low vitamin D levels (26.1 ng/mL).

Cytological analysis of a bone marrow sample obtained by aspiration revealed a proportion of mast cells of less than 5% (3%), with a predominance (80%) of bone marrow mast cells with an abnormal morphology (elongated oval nucleus and hypogranular cytoplasm). Immunophenotypic analysis performed by multiparameter flow cytometry revealed mast cells expressing CD25 (0.1%). These results together with the high total tryptase level were compatible with a diagnosis of indolent systemic mastocytosis. The final diagnosis was anaphylaxis due to sensitization to wasp venom and secondary anaphylaxis to *Megachile* sting in a patient with indolent systemic mastocytosis.

Leaf-cutter bees (Apoidea: Megachillidae) are solitary bees which use leaf pieces for constructing cells, making use of pre-existing cavities for their nests. They are therefore frequently located in manmade structures, such as brick constructions and walls [7]. In general, leafcutter bees are not considered aggressive, and sting only when handled or threatened.

The only report of allergy to *Megachile* bees is related to inhalant allergy in insect rearing facilities where *Megachile rotundata* bees are produced for use as pollinators [8]. To our knowledge, however, allergic reactions to Megachilidae stings have not been reported and no venom proteins have been described. However, severe reactions to other solitary bee stings were found by Pence et al [9] on studying 13 patients with severe reactions to stings by sweat bees (Apoidea: Halictidae).

Although allergy to leaf-cutter bee venom is unknown, we suggest the possibility that minor hymenoptera species could be responsible for anaphylaxis or other severe reactions in patients with mastocytosis, as has been suggested for patients with honeybee and wasp venom allergy [10]. Further investigations are needed to obtain standardized extracts of solitary bees for a complete diagnosis.

Acknowledgments

We would like to thank Dr. Francisco Javier Ortiz Sánchez for his help identifying the species.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

V. Iraola is employee of Laboratorios LETI. The other authors declare that they have no conflicts of interest.

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Manuscript received April 25, 2013; accepted for publication, July 3, 2013.

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Prediction of the Evolution of Common Variable Immunodeficiency: HLA Typing for Patients With Selective IgA Deficiency

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Key words: Common variable immunodeficiency. Selective IgA deficiency. Human leukocyte antigen.

Palabras clave: Immunodeficiencia común variable. Deficiencia selectiva de IgA. Antígeno leucocitario humano.

Selective IgA deficiency (SIgAD) is the most common primary immunodeficiency. It is characterized by serum IgA levels <0.5 mg/dL with normal IgG and IgM in a patient aged >4 years [1,2].

Common variable immunodeficiency (CVID) is a primary antibody deficiency that is characterized by a lack of immunoglobulin (Ig) production, specific antibody deficiency, and recurrent infections of unknown etiology [3-5]. Symptomatic SIgAD shares many traits with CVID, and some patients with SIgAD progress to CVID [4, 6]. It has been suggested that association between human leukocyte antigen (HLA) A1, B8, DR3, DQ2, or any part of this haplotype and IgA deficiency could indicate progression to CVID [4, 6].

The first patient was a 10-year-old boy who was referred to our hospital in 2004 because of frequent sinopulmonary and gastrointestinal infections. The laboratory workup revealed a low serum IgA level (<9 mg/dL) with normal IgM and IgG levels; therefore, he was diagnosed with SIgAD. Specific antibody production against polysaccharide and polypeptide antigens was defective (Table). Despite antibiotic prophylaxis during the following 5 years, he experienced recurrent episodes of lower respiratory tract infection and otitis media.

Table. Clinical Data of Patients

Parameter	Case 1	Case 2
Sex	Male	Female
Current age, y	10	18
Age at onset of disease, y	2	6
Age at diagnosis, y	2	11
Respiratory tract infections	+	+
Chronic diarrhea	+	+
Autoimmunity	Autoimmune hemolytic anemia	Autoimmune hemolytic anemia, celiac disease, Hashimoto thyroiditis
IgG, mg/dL	2000	780
IgA, mg/dL	IgA <9	5
IgM, mg/dL	151	69
Antidiphtheria, IU/mL	0.06	0.1
Antitetanus, IU/mL	0.1	0.3
Antipneumococcus before vaccination, IU/mL	5.7	8.4
Antipneumococcus after vaccination, IU/mL	7.5	129.0
White blood cells, cells/mm^3	7800	5400
Lymphocytes, %	68	47
Absolute T-cell count, cells/mm^3	3394	1903
Absolute B-cell count, cells/mm^3	477	185
Cytotoxic T-cell count, cells/mm^3	2917	761
Helper T-cell count, cells/mm^3	265	634
Regulatory T cells, % of total CD4$^+$ T cells	2.33	0.72
HLA typing	A*1101, A*3101/B*1301, B*52/ Cw*0302, Cw*1202 DRB1*1201/DRB1*15/DQB1*0301/ DQB1*0602/ DQA1*0102/ DQA1*0501	A*3001, A*6801 /B*1302,B*27/ Cw*0102, Cw*0602 DRB1*0701, DRB1*1101/ DQB1*0201, DQB1*0301 DQA1*0201, DQA1*0501

At the age of 7, he was admitted to hospital because of early fatigue, dyspnea, and chronic cough. Analysis of lung biopsy was compatible with lymphocytic interstitial pneumonitis. Unfortunately, the patient did not respond to treatment and was readmitted with the same symptoms 6 months later. A chest high-resolution computed tomography (HRCT) scan revealed tubular bronchiectasis. During this admission, the patient became pale and the color of his urine darkened. Hematological tests revealed a low hemoglobin level, with a high percentage of reticulocytes; the result of the direct Coombs test was positive. A diagnosis of autoimmune hemolytic anemia was made. Consequently, and because of the respiratory complications, we decided to administer intravenous immunoglobulin (IVIG).

The patient also had diffuse scaly pityriasis versicolor–like macules on his chest and abdomen. Histopathology of a skin biopsy specimen was consistent with epidermodysplasia verruciformis suggesting defects of innate immunity.

Given the unusual presentation of this case, further immunologic workups were performed and revealed abnormal subsets of cellular immunity, such as a decreased regulatory T-cell population (2.33% of total $CD4^+$ T cells [<2SD of normal population, 2.36%]). B-cell class switch recombination was also anomalous ($IgD^-27^+CD19^+$ switched memory B-cell percentage of peripheral blood lymphocytes was 0.38% [<2SD of normal population, 0.4%]). Accordingly, HLA typing was performed (Table). Because of the need for regular IVIG, the patient's Ig levels were evaluated monthly for 3 years. Surprisingly, values decreased, and, finally, in 2012, the diagnosis of CVID was confirmed (IgM, 20 mg/dL; IgA, 4 mg/dL; IgE, 9 mg/dL; IgG, 500 mg/dL).

The second patient was an 18-year-old woman whose medical problems began at the age of 6 years with respiratory tract infections. Following recurrent episodes of severe pneumonia, she was referred to our hospital for an immunology workup, which revealed SIgAD. She developed specific antibody protection after pneumococcal vaccination. She had progressively developed chronic lung disease during previous years, and her HRCT scans revealed bronchiectasis. Therefore, closely monitored IVIG was started to manage this condition. Within the following 2 years, she was diagnosed with Hashimoto thyroiditis. She also had recurrent diarrhea in the absence of any detectable bacterial or parasitic infection. Following a gastrointestinal biopsy, she was diagnosed with celiac disease. At the age of 15 years, she was admitted to hospital with fever, early fatigue, and lethargy. Ultrasonography revealed hepatomegaly and splenomegaly, and she was diagnosed with autoimmune hemolytic anemia. During 7 years of follow-up, she developed signs and symptoms of asthma, allergic rhinitis, and atopic dermatitis. HLA typing (Table) and further immunologic workups revealed a low regulatory T-cell percentage (0.72% of total $CD4^+$ T cells) and switched memory B-cell percentage (0.28% of peripheral blood lymphocytes), similar to those of the patient described above. Despite monthly IVIG therapy, serum Ig levels, especially IgG, declined gradually, and the patient was considered to have CVID.

Although HLA markers are the principal genetic risk factor associated with SIgAD and CVID, evaluation to map causal variants through the distinct haplotypes was unsuccessful [7]. Whereas 10% to 15% of patients with CVID have first-degree relatives with SIgAD, it is suggested that shared genetic factors influence disease susceptibility [8,9]. Investigation of shared HLA markers could elucidate the underlying genetic causes and may be predictors of the progress of SIgAD to CVID in patients with severe clinical manifestations. We present 2 cases of SIgAD with a similar and specific presentation, including autoimmune hemolytic anemia, bronchiectasis, polyclonal lymphocytic infiltrations, deficiency of specific antibodies against T-independent antigens, and reduced class-switched memory B-cell and regulatory T-cell percentages. HLA typing of these cases revealed a common MHC class 2 haplotype (DQB1*0301, DQA1*0501). Although the HLA-DQB1*0301 allele has a protective effect against SIgAD, we observed that the first patient had another allele (DQB1*0602) that was negatively associated with SIgAD. This finding is consistent with those of previous analytic studies of HLA, which revealed unusual HLA markers in SIgAD patients who were predisposed to progress to CVID. We previously reported on 4 SIgAD patients with different ancestries who presented autoimmune disorders and IgG subclass deficiency that progressed to CVID. These selected patients had the same HLA alleles as the 2 patients we describe here. Patient 2 was heterozygous for DR7, DR11, DQ2, and DQ3; patient 1 was heterozygous for DQ3 [4].

Differences in HLA alleles/haplotypes between SIgAD and CVID and the presence of protective HLA markers in SIgAD patients who progressed to CVID suggest that the genetic association in most CVID patients differs from that of patients with permanent SIgAD [10].

In conclusion, SIgAD patients with severe clinical manifestations, including autoimmune processes, infections, and specific antibody deficiency can progress to CVID and be candidates for early IVIG therapy. HLA typing may be helpful for prediction of progression to CVID.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Manuscript received April 10, 2013; accepted for publication July 8, 2014.

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Successful Treatment of Systemic Cytomegalovirus Infection in Severe Combined Immunodeficiency Using Allogeneic Bone Marrow Transplantation Followed by Adoptive Immunotherapy

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Key words: Cytomegalovirus. Severe combined immunodeficiency. Bone marrow transplantation. Donor lymphocyte infusions.

Palabras clave: Citomegalovirus. Inmunodeficiencia combinada severa. Transplante de médula. Infusiones de linfocitos del donante.

Severe combined immunodeficiency (SCID) is one of the most severe forms of primary immunodeficiency disease. Although infants with SCID generally appear healthy at birth, they are unable to clear infections during the first few months of life. The presence of insidiously progressive respiratory disease with radiological evidence of interstitial pneumonia suggests the involvement of *Pneumocystis jiroveci* or cytomegalovirus (CMV) infection.

Hematopoietic stem cell transplantation (HSCT) and gene therapy are the curative treatments of choice for patients with SCID. Outcome is poor in those with ongoing *P jiroveci* or CMV infection. Adoptive immunotherapy with CMV-specific cytotoxic T lymphocytes (CTL) has recently been used for post-HSCT patients with refractory CMV infections [1,2]. However, this therapy is labor-intensive and expensive. Therefore, it is only used in a limited number of institutions. Conversely, activated $CD4^+$ T cells can be easily expanded ex vivo and used for adoptive immunotherapy against cancer or as a component of therapy based on donor lymphocyte infusion (DLI) [3-5]. Numazaki et al [6] reported the case of an infant diagnosed with severe interstitial pneumonia associated with CMV infection who was successfully treated with adoptive immunotherapy using activated $CD4^+$ T cells.

We report the case of a patient with X-linked SCID and systemic CMV infection. Treatment involved bone marrow transplantation (BMT) from a human leukocyte antigen (HLA)–matched sibling donor and adoptive immunotherapy administered by infusion of CMV-positive donor-derived activated $CD4^+$ T cells. This combination successfully cured CMV infection.

A 3-month-old Japanese boy was admitted to a local hospital because of long-standing severe cough. The laboratory studies revealed lymphopenia, and the patient was transferred to Toyama University Hospital, Toyama, Japan owing to the possibility of primary immunodeficiency disease. Physical examination revealed fever, tachypnea, respiratory retraction, and hepatomegaly. The laboratory studies revealed lymphopenia (378/μL), thrombocytopenia (36 x 10^3/μL), anemia (hemoglobin, 7.7 g/dL), hypoproteinemia (total protein, 4.0 g/dL), elevated liver enzymes (aspartate aminotransferase, 564 IU/L; alanine aminotransferase, 91 IU/L; lactate dehydrogenase, 1088 IU/L), and hypogammaglobulinemia (IgG, 85 mg/dL; IgA, 1 mg/dL; IgM, 29 mg/dL). The common γ chain was not expressed on lymphocytes, and the genetic analysis revealed a novel 609delG mutation in *IL2RG*. Therefore, the patient was diagnosed with X-linked SCID. A chest radiograph showed interstitial shadow, and the thymus was not visible. Thoracic computed tomography images demonstrated consolidation in the right lower lobe and diffuse ground-glass opacities in both lungs; the thymus was not visible. Tests for *P jiroveci, Aspergillus* species, and *Candida* species were all negative. However, the test for CMV antigenemia was positive (C7-HRP, 34/44 000). In addition, CMV-DNA was detected in all the samples analyzed, including blood, urine, sputum, cerebrospinal fluid, and stool. Taken together, the data indicated systemic CMV infection.

The patient was immediately treated with intravenous immunoglobulin, ganciclovir, trimethoprim-sulfamethoxazole, and sivelestat sodium. However, a pulmonary hemorrhage and hypoventilation due to severe CMV pneumonia required mechanical ventilation. Although methylprednisolone pulse therapy and nitrogen oxide inhalation were also administered for CMV pneumonia, the patient's respiratory condition worsened. On the 13th day of hospitalization, the patient underwent BMT from an HLA-matched sibling without conditioning. Ciclosporin A and methylprednisolone were administered as prophylaxis for graft-versus-host disease.

The patient's respiratory condition gradually improved, and he was taken off the mechanical ventilator on day 8 after BMT (Figure). The donor was positive for anti-CMV antibody and had CMV-specific $CD8^+$ T cells (0.02%). Before BMT, activated $CD4^+$ T cells were prepared from the donor to use in the treatment of severe CMV infection. Although the patient was not under mechanical ventilation at this stage, he still required oxygen, and his CMV-DNA copy number was high. Therefore, he was treated with foscarnet beginning on day 18 after BMT for possible ganciclovir-resistant CMV infection. He also received CD4-DLI (5 x 10^7 cells) on days 24 and 38 after BMT. CMV-DNAemia had disappeared by day 39 after BMT. Although the patient has a mild developmental delay, he is doing well without intravenous immunoglobulin (replacement).

Various types of infection have been observed in patients with SCID. One of the major problems affecting these patients is interstitial pneumonia due to *P jiroveci* or CMV infection. CMV-induced pneumonia may be more severe and leads to a

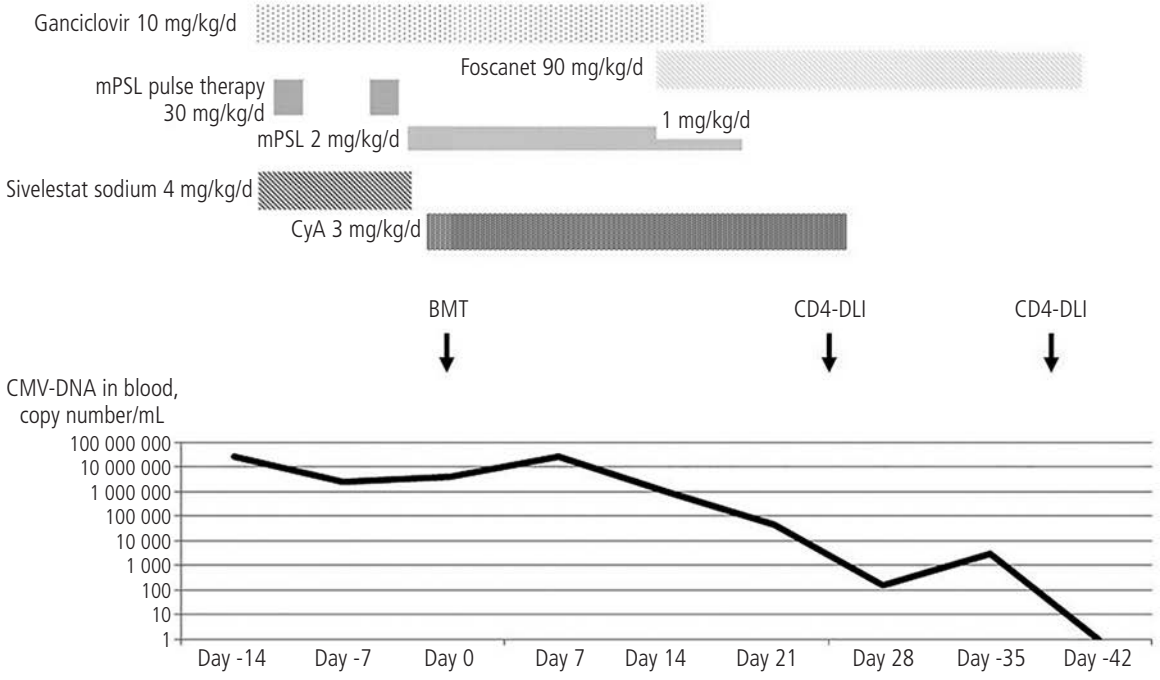

Figure. Clinical course of the patient. The CMV-DNA copy number decreased after BMT followed by CD4-DLI. mPSL indicates methylprednisolone, CyA, ciclosporin A; CMV, cytomegalovirus; BMT, bone marrow transplantation; DLI, donor lymphocyte infusion.

poor outcome. The donor CMV-specific CTLs were transfused to the patient during allogeneic BMT and may have begun to act against CMV infection. CD4-DLI therapy on days 24 and 38 after BMT may also have contributed to the control of CMV infection.

CMV infection is a major complication of allogeneic HSCT. Ganciclovir is an effective antiviral agent, and cidofovir and foscarnet are also effective for CMV infection in ganciclovir-resistant recipients. CMV infection is resistant to these antiviral drugs in certain patients, especially those receiving T-cell–depleted HSCT and those with primary immunodeficiency disease [7]. Treatment with CMV-specific CTLs after HSCT has resulted in cellular immune reconstitution and suppression of viremia [8,9]. The first adoptive immunotherapy involved the use of CMV-specific CTL clones generated by stimulating donor T cells with CMV-infected skin fibroblasts [8]. Dendritic cells and Epstein-Barr virus–transformed lymphoblastoid cell lines have also been used as antigen-presenting cells for expansion of CTLs [9,10]. However, these methods may not be suitable for clinical contexts that require rapid generation of CTLs.

Expansion of autologous T cells from peripheral blood mononuclear cells ex vivo is a feasible and efficacious approach [3] that rapidly enabled complete chimerism to be achieved without graft-versus-host disease in a patient with primary immunodeficiency disease [5]. We performed CD4-DLI for refractory CMV infection in a patient with X-linked SCID. Activated $CD4^+$ T lymphocytes might have a bystander effect for donor-derived CMV-specific CTLs that were transfused during BMT.

In conclusion, we report the case of a patient with X-linked SCID associated with severe CMV infection. The patient recovered from severe CMV infection with CMV-specific CTLs from an HLA-matched sibling donor and adoptive immunotherapy with CD4-DLI.

Acknowledgments

We thank Chikako Sakai, Hitoshi Moriuchi, Shizuko Minegishi, Junko Michino, Tomoko Toma, Noriko Nakagawa and Kimiyasu Shiraki for their excellent technical assistance. We are also grateful to the medical and nursing staff for taking care of the patient.

Funding

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology and grants from the Ministry of Health, Labour and Welfare of Japan.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Manuscript received April 24, 2013; accepted for publication July 8, 2013.

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Proteins Responsible for Nut Allergies

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Key words: Nut. Allergy. Proteins.

Palabras clave: Frutos secos, Alergia, Proteínas.

Food allergy diagnoses have increased notably in recent years thanks to, among other reasons, a greater understanding of the proteins involved. Plant allergies, and in particular, allergies to nuts are the leading cause of food allergy in adults, together with fruit allergies [1]. Plant allergies in a given population are related to consumption habits and pollen patterns in the region under study.

The objective of this study was to determine the proteins responsible for nut allergies in Asturias, a region situated on the Cantabrian Sea with an Atlantic climate and a different pollen representation to the rest of Spain. We recruited patients diagnosed with nut allergy from a database of patients seen at an outpatient clinic between 1990 and 2012. Inclusion criteria were typical clinical manifestations of IgE-mediated allergy to 1 of 4 widely eaten nuts (hazelnut, almond, peanut or walnut) and a positive skin prick test (ALK) or prick to prick test to any of the 4 nuts. The allergens responsible for the most severe reactions were determined as follows: Cor a 1 and 8 for hazelnut, Ara h 2, 8 and 9 for peanut, and Jug r1 and 3 for walnut (ImmunoCAP, ISAC 112; Thermo Fisher Scientific). Finally, a skin prick test was performed with pollen from grasses (Lolium), birch, profilin and lipid transfer protein (LTP) (peach) (ALK).

Thirty-seven patients (20 women) with a mean age of 26 years (interquartile range, 24-45 years) were studied. Hazelnut was the most common trigger in 25 patients (68%), walnut in 19 (51%), peanut in 15 (41%), and almond in just 1 case (3%). Symptoms included oral allergy syndrome (OAS) in 22 cases (59% of patients), anaphylaxis in 10 (27%) and urticaria/angioedema in 8 (22%). Seventy percent of patients showed sensitization to grass pollen and 56% to birch pollen.

As for the proteins responsible, Bet v 1 homologs (PR-10) were identified in 18 cases (50%) and LTPs (40%) in 15. One patient was found to be sensitized to Ara h 2 and another to Jug r 1. In 2 cases the protein responsible was not identified.

Table. Relationship Between Proteins Responsible for Nut Allergy and Clinical Manifestations[a]

	OAS	Systemic Manifestations
Bet v 1 homologs	14	4
LTPs	6	9

Abbreviations: LTPs, lipid transfer proteins; OAS, oral allergy syndrome.
[a]Results shown as number of patients.

The relationship between the type of symptoms and the triggering protein is shown in the Table. Sensitization to Bet v 1 homologs tended to be manifested in the form of OAS, while that produced by LTPs led to more systemic reactions (P=.038).

With regard to the type of nut and protein responsible for the clinical picture, patients allergic to hazelnuts were significantly more likely to be sensitized to Bet v 1 homologs, whilst those allergic to peanuts were more likely to be sensitized to LTPs (P=.041).

In conclusion, we have shown that in a sample of patients allergic to nuts in the north-western region of Spain the main proteins responsible are the Bet v 1 homologs and LTPs. In the case of hazelnuts, this would represent an intermediate position between central and northern Europe, where PR-10 predominates, and southern Europe, where LTPs predominate [2]. In Asturias, the pollens of greatest interest from an allergological perspective are clearly grasses and to a much lesser extent Betulaceae pollen (birch, hazelnut tree, and alder). Nevertheless, the presence of pollen from Betulaceae would explain the results described. Hazelnut was the most frequently involved nut in our series, like in other series in central and northern Europe. By contrast, studies in other Mediterranean regions in Spain have found walnuts and almonds to be the most frequent causes of nut allergies [3,4]. Furthermore, it is worth noting that hazelnut trees are common in Asturias and consumption of hazelnuts is high. Finally, it should be noted that although the most common clinical manifestation of nut allergy in our series was OAS, half of the patients also presented systemic reactions.

Acknowledgments

We would like to thank nurses Maria Luisa Lago Alvariño and Maria Luisa Vélez Jórganes for their help.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Manuscript received June 13, 2013; accepted for publication, July 10, 2013.

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Analysis of the IgE Response to Pine Nut Allergens in Italian Allergic Patients

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Key words: Pine nut allergy. Food allergy. Anaphylaxis.

Palabras clave: Alergia a piñones. Alergia alimentaria. Anafilaxia.

Plant-derived foods are the main cause of food allergy in Italian adults, and pine nut seems to be a relevant allergen. Allergy to pine nut is frequently severe [1] and can affect children. Patients can become sensitized through airborne transmission [2]. Pine nuts are found in sweets, cookies, and pasta seasoned with pesto sauce, yet little is known about pine nut allergens. Several proteins of different molecular weights (17 kDa [3]; 30, 44, and 50 kDa [4,5]; and <14 kDa [5]) have been detected. A 6-kDa albumin and a 50-kDa vicilin were recently shown to be major allergens [6], and surprisingly uniform IgE reactivity to 5 allergens ranging between 6 kDa and 47 kDa was detected in 5 children [7]. We investigated the main pine nut allergens in Italian patients.

Pine nut–allergic patients were identified at 33 Italian allergy centers in 2011. Diagnosis was based on a clinical history of oral allergy syndrome, asthma, urticaria/angioedema, and/or anaphylaxis following the ingestion of pine nuts in any form (ie, raw, cooked, and roasted) associated with a positive skin prick test result with fresh pine nut and/or a commercial extract (Stallergènes or Lofarma). We excluded patients sensitized to cross-reacting plant-food allergens such as type 10 pathogenesis-related protein, profilin, and lipid transfer protein (diagnosed by skin prick testing with commercial birch pollen extract, profilin-enriched date palm pollen extract [ALK-Abelló], and peach extract [ALK-Abelló]). Patients were interviewed to ascertain their clinical reactivity to other foods, particularly walnut, hazelnut, almond, peanut, sesame seed, Brazil nut, and sunflower seed.

Eight grams of pine nuts defatted with hexane were extracted for 1 hour in 100 mL of 0.9 M NaCl at 4°C with

stirring. After centrifuging, the supernatant was harvested and dialyzed against saline. The protein content was 1.24 mg/mL. The lipophilic fraction was obtained by evaporation of the hexane used to de-fat the nuts. Electrophoresis of pine nut extract (25 µg/lane) was carried out in a precast 10% polyacrylamide Nupage Bis-Tris gel (Invitrogen) at 180 mA for 1 hour under both reducing and nonreducing conditions. The resolved proteins were transferred onto a nitrocellulose membrane according to Towbin et al [8]. The membrane was saturated in Tris-buffered saline buffer containing 5% defatted dry milk (saturating buffer) and incubated with patients' or normal serum diluted 1:5 in saturating buffer. Bound specific IgE was detected using peroxidase-conjugated antihuman goat IgE as a substrate (1:8000; BiosPacific) and the ECL Western blotting kit (Amersham).

The study sample comprised 12 patients (aged 3-71 years; 3 males). Ten had a history of allergy to pine nut only, 1 to walnut, and 1 to walnut, hazelnut, and peanut. Seven patients experienced pine nut–induced anaphylaxis, 3 urticaria/angioedema, 1 asthma, and 1 laryngeal edema. Two girls aged 12 and 15 had not eaten pine nuts for 10 and 6 years, respectively, after experiencing pine nut–induced anaphylaxis. Given the severity of the reported reactions, confirmatory food challenges were not performed.

Sera from 11 patients were available for in vitro studies. Seven patients (all monosensitized to pine nut) showed IgE reactivity on the immunoblot. Four patients (patients 2, 4, 5, and 11) scored positive under reducing conditions against a spectrum of proteins with different molecular weights. One reacted at about 17 kDa only, and 1 at <14 kDa only. The remaining 2 patients had a more complex profile: one showed IgE reactivity at < 14, 40, and 48 kDa and the other at <14, 30, 35, 45, and approximately 60 kDa (Figure, A). Analysis of sera from 5 patients (patients 2, 5, 6, 7, and 11) based on immunoblotting under nonreducing conditions was positive (Figure, B). In this set of experiments, 3 patients had an almost identical profile with IgE reactivity to proteins at 14, 35, and 40 kDa, and slight reactivity at higher molecular weights in 2 cases. One patient showed IgE reactivity at 17 kDa only, and another showed slight reactivity at 40 kDa only. Only 3 sera (patients 2, 5, and 11) reacted under both reducing and nonreducing conditions. The IgE reactivity profile remained unchanged in 2 cases (patients 2 and 11) but changed significantly in patient 5, with disappearance of reactivity at low molecular weights. Two patients who scored negative under reducing conditions showed strong IgE reactivity under nonreducing conditions (patients 6 and 7). Serum from patient 3 (history of 2 pine nut–induced anaphylactic episodes and negative results in skin prick testing, ImmunoCAP, and immunoblot with aqueous extract under both reducing and nonreducing conditions) produced a frankly positive IgE response at about 17 kDa and 30-40 kDa when tested against the lipophilic fraction of pine nut extract (Figure, C), suggesting exclusive hypersensitivity to fat-soluble proteins.

Primary pine nut allergy is uncommon (only 12 cases were found in 33 Italian allergy clinics in 1 year) and severe (anaphylaxis occurred in 7 of the 12 cases) and can affect all age groups (age at onset ranged between 3 and 71 years). Sera from 5 patients did not react on immunoblot, possibly owing to low specific IgE levels; indeed, 2 had not ingested pine nuts for 6 and 10 years. Among the reactors, the IgE profile varied widely, with bands ranging from 6 kDa to 90 kDa. In several cases, the molecular weights corresponded to those of the allergens reported [3-7]. Interestingly, IgE reactivity varied widely depending on whether immunoblot analysis was carried out under reducing or nonreducing conditions, suggesting that reduction may alter some pine nut allergens, thus preventing their recognition by specific IgE. This difference in analysis conditions may explain the variations in findings from previous studies of pine nut allergy [3-7]. Furthermore, serum from a patient whose skin and serological test results were negative showed IgE reactivity against allergens present in the lipophilic

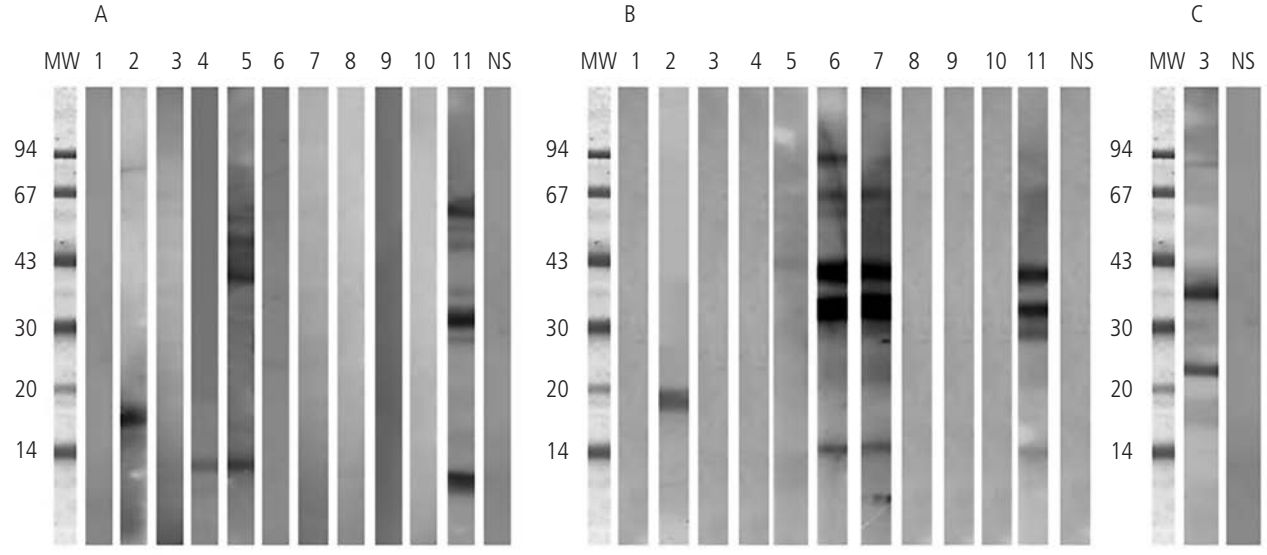

Figure. Immmunoblot analysis of sera from 11 pine nut–allergic patients. A, Reducing conditions. B, Nonreducing conditions. C, Immunoblot using the lipophilic fraction of pine nut extract with serum from a patient with a history of pine nut–induced anaphylaxis whose results were negative in conventional in vivo and in vitro tests. MW indicates molecular weight markers; NS, normal control serum.

fraction of the pine nut extract. Similar phenomena have been observed in patients who are allergic to nuts, seeds, or both [9,10]. In conclusion, pine nut–allergic patients may react to several allergens, most of which can induce systemic symptoms. Pine nut allergens have different physical and chemical features that may considerably influence both in vivo and in vitro diagnosis.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Manuscript received January 7, 2013; accepted for publication July 10, 2013.

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Allergy to Red Meat in Adulthood: A Case Report

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Key words: Anaphylaxis. Red meat. Cross-reactivity. Carbohydrate antigen. Galactose-α-1,3-Galactose.

Palabras clave: Anafilaxia. Carne roja. Reactividad cruzada. Epítopo de carbohidrato. Galactosa-alfa-1,3-galactosa.

Meat allergy is rare in adults [1]. The major allergens involved in mammalian meat allergy are serum albumins and immunoglobulins [1,2]. Systemic reactions to a new mammalian cross-reactive carbohydrate determinant, galactose-α-1,3-galactose, have been described [3]. We report a case of food allergy to red meat that developed in late adulthood.

A 66-year-old man was referred to the immunoallergology department of our hospital with a 2-year history of recurrent episodes of anaphylaxis. He had experienced 4 similar episodes (generalized urticaria and angioedema, nausea, vomiting, diarrhea, hypotension, and dizziness) after ingestion of pork, goat, lamb, and rabbit. These reactions were not immediate and occurred up to 3 hours after ingestion. The patient attended the emergency department for each episode. He lost consciousness in 2 of the 4 episodes, and intramuscular adrenaline was administered.

After the first reaction, the patient suspected that he was allergic to all kinds of meat (even bird meat); therefore, he eliminated meat from his diet. However, he ate mammalian meat on 3 social occasions and experienced a reaction each time. Before the first reaction, the patient ate mammalian meat regularly, with no complaints.

On admission to our department, 2 months after the most recent episode of anaphylaxis, physical examination was unremarkable. The patient had no previous history of atopy or allergy or intolerance to food or drugs. Malaria in childhood was the only relevant pathologic finding in his past medical history. The patient was born in Kenya and lived there until the age of 17. He moved to Coimbra, Portugal, where he lived until the age of 32, when he moved to Mozambique. At age 34, he returned to Coimbra and has lived on a farm since then. He has also had daily contact with dogs and cats since childhood. The patient denied having had tick bites in the last year.

The results of laboratory studies (full blood count, erythrocyte sedimentation rate, serum electrolytes, liver and renal function tests, baseline complement and serum tryptase levels, and skin biopsy) were all normal. The patient had a high level of total IgE (258 IU/mL). Skin prick tests using commercial extracts of common aeroallergens and food

allergens (milk, egg, and bird and mammalian meats [Bial-Aristegui]) were performed as previously described [4]; the results were positive for dog dander (mean wheal diameter, 5 mm) and various mammalian meats: lamb (4 mm), rabbit (4 mm), pork (3 mm), and beef (5 mm).

Specific IgE to mammalian dander (cat, dog, pig), meat (lamb, rabbit, pork, beef), and bovine serum albumin was measured using the ImmunoCAP FEIA system (Thermo Fisher). Levels above 0.35 kU_A/L were considered positive. Specific IgE determinations were positive for dog dander (0.60 kU_A/L) and for pork and beef (2.72 kU_A/L and 2.98 kU_A/L, respectively).

SDS-PAGE immunoblotting was performed with extracts from raw and cooked pork and beef to investigate the sensitization profile. A similar IgE binding pattern was observed for both extracts (Figure). IgE binding activity seemed to be more intense when cooked extracts were used. In addition, SDS-PAGE immunoblotting with other cooked mammalian and bird meats was also performed. As shown in the Figure, IgE binding seems to be specific to the mammalian meats, as no binding was detected with the bird meat extracts (chicken, turkey, and duck). Therefore, we thought that specific IgE against the galactose-α-1,3-galactose epitope could be involved in the patient's anaphylaxis, as this carbohydrate moiety is typical of nonprimate mammalian proteins. Accordingly, we determined specific IgE to galactose-α-1,3-galactose, as previously described [5], and found a very high value (42.4 kU_A/L [Thermo Fisher]).

The patient was advised to continue avoiding red meat, although poultry was allowed. Patient education included detailed information on the risk of cross-contamination, particularly when eating out. A written emergency plan including self-injectable adrenaline, cetirizine, and prednisolone was prepared. The avoidance measures ensured that the patient experienced no further anaphylactic episodes.

The main allergens in meats are serum albumins and immunoglobulins. Both can be significantly changed by industrial processing and cooking, which decrease allergenicity [2,6]. Meat allergy presenting in infancy is normally outgrown during the first years of life and is very rare in adults [1].

The patient described here became allergic to meat later in life. In contrast with the severity of the allergic reaction, the wheal response to prick tests with meat extracts was very weak (<5 mm to various mammalian meats). This finding was consistent with those of other cases described in the literature [3,7,8]. Moreover, the patient had a low level of specific IgE to pork and beef compared with the value for specific IgE to galactose-α-1,3-galactose. As suspected, and consistent with cases described elsewhere, the delayed reaction to red meat was associated with sensitization to an allergic determinant, namely, galactose-α-1,3-galactose [7,8,9].

The oligosaccharide galactose-α-1,3-galactose is involved in delayed anaphylactic reactions to red meats, such as beef, pork, lamb, and rabbit [3,7], but it is not detected in poultry or fish.

The galactose-α-1,3-galactose epitope is abundantly expressed in cells and tissues of nonprimate mammals, and production of specific IgE against this epitope was reported in patients with allergy to red meat [3,9].

We did not find it essential to perform oral challenge tests to red meat to confirm the diagnosis owing to the consistent clinical history, that is, episodes were always associated with ingestion of red meat, specific IgE was positive to galactose-α-1,3-galactose and the patient was asymptomatic when he did not eat meat. The role of food challenge in delayed reactions to mammalian meat is still under investigation and has yet to be defined [9]. The

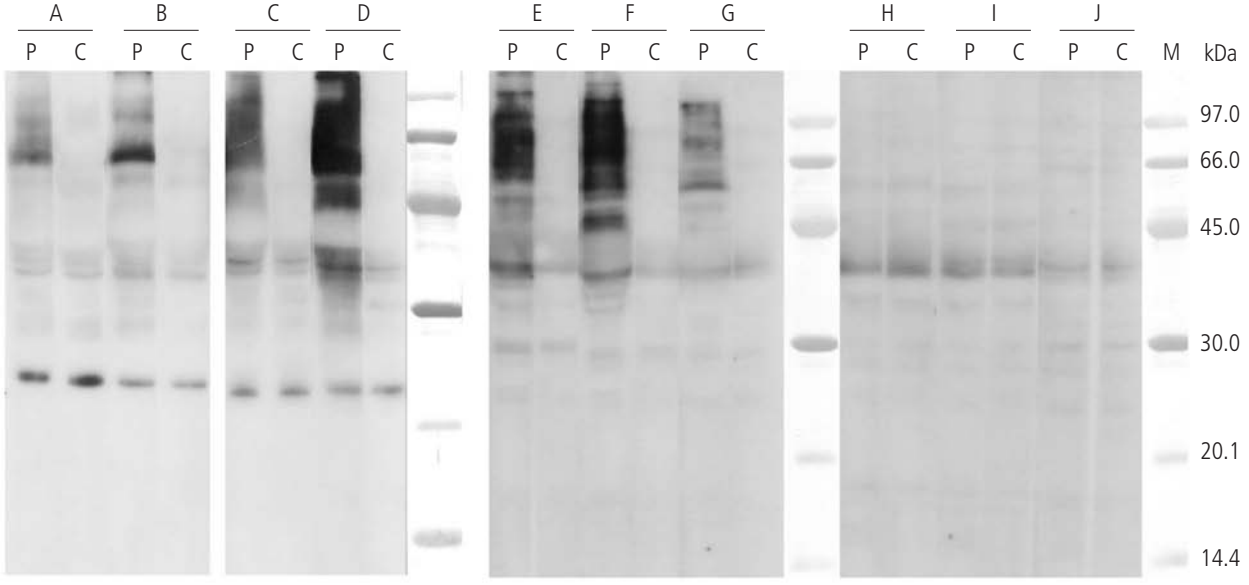

Figure. SDS-PAGE Immunoblotting results. A, raw pork; B, cooked pork; C, raw beef; D, cooked beef; E, cooked colt meat; F, cooked lamb; G, cooked rabbit meat; H, cooked chicken meat; I, cooked turkey meat; J, cooked duck meat. Lane P, patient's serum; lane C, control serum (pool of sera from nonatopic individuals); lane M, molecular mass marker.

pathophysiology of the syndrome has not been clarified, and more studies are required to explain why the reactions are delayed and the mechanisms responsible for the production of IgE antibodies to galactose-α-1,3-galactose [9]. A very high prevalence of IgE to galactose-α-1,3-galactose has been reported among parasite-infected patients from Central Africa [10]. Importantly, our patient lived in Kenya for many years and had malaria as a child. We could question whether this parasitic infection, or perhaps some other infection, may have played a role in the sensitization process, despite the late occurrence of the first episode of anaphylaxis.

According to the literature, the clear positive result of serum specific IgE against galactose-α-1,3-galactose supports the involvement of this cross-reactive carbohydrate in the case of allergy to red meat we report [3,9].

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

B Bartolomé is an employee of Bial-Aristegui, Bilbao, Spain. The remaining authors declare that they have no conflicts of interest.

Previous Presentation

The data reported in this study were presented in abstract form and poster form at the 2011 meeting of SEAIC (Alergia a la carne de mamíferos en la edad adulta. I Carrapatoso, B Bartolomé, F Ribeiro, J Martínez, A Segorbe Luís. JIACI 2011;21 [Suppl 4]:272).

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Manuscript received March 26, 2013; accepted for publication July 16, 2013.

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Pork-Cat Syndrome as a Cause of Occupational Asthma

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Key words: Pork. Cat. Pork-cat syndrome. Asthma. Occupational asthma.

Palabras clave: Cerdo. Gato. Síndrome gato-cerdo. Asma. Asma ocupacional.

Pork-cat syndrome is an infrequent entity. It is characterized by hypersensitivity to cat serum albumin, which cross-reacts with porcine serum albumin owing to antigenic similarity between the two. Pork-cat syndrome has been reported to be the cause of severe reactions after the ingestion of pork meat [1]. Given the structural differences between mammal serum albumins, considerable variability in cross-reactivity patterns between mammals has been reported [2]. Published data suggest that sensitization to cat dander precedes the development of pork allergy, which occurs later in life, often during adolescence or adulthood [3].

We report the case of a 30-year-old woman with a family history of atopy. At the age of 13, soon after receiving a cat, she began to experience perennial ocular and nasal symptoms, as well as cough, wheeze, and dyspnea, all of which worsened in spring. At age 14, she was diagnosed with allergic rhinoconjunctivitis and mild intermittent asthma caused by pollen and dander. Her allergy improved when the cat was removed, and symptoms remained limited to mild rhinoconjunctivitis in springtime, although she still had mild asthma throughout the year when practicing sports.

When she was 20, she started working at a grocery store selling cured meats. One of her duties was to cut pork bones (cured and cooked ham). From that point, her rhinoconjunctivitis worsened and her asthma attacks resumed. These manifestations were exacerbated at her workplace, especially when she had to cut pork bones. Her asthma continued to deteriorate, and she experienced 3-4 exacerbations per year that forced her to be absent from work. She frequently needed aerosol therapy after work, although she felt better on days off work and was almost asymptomatic during vacations. In 2010-2011, she was admitted to hospital on 4 occasions, forcing her to miss work for 1-2 months each time. Her asthma was controlled with oral corticosteroids.

Since the patient experienced pruritus and erythema when she handled meat products, she used protective gloves at work. However, on one occasion, a scratch with a pork bone resulted in marked local swelling and pruritus. She avoided eating pork meat owing to sudden onset of dyspepsia, although she tolerated cured meats. She also tolerated well-done beef. In 2007, she bought a dog, which had to be removed after 15-20 days because of rapid-onset asthma attacks.

The results of a basic blood analysis and chest radiograph were normal, except for the eosinophil count (900/mm^3). Baseline spirometry revealed the forced expiratory volume in 1 second (FEV_1) to be 1.70 L, forced vital capacity (FVC) 2.94 L, FEV_1/FVC 57.77%, peak expiratory flow 3.83 L/s, and forced expiratory flow, midexpiratory phase 0.79 L/s. A methacholine challenge test performed while the patient was working revealed that the provocative dose of methacholine required to cause a 20% reduction in FEV_1 (PC_{20}) was 0.05 mg/mL. The test was repeated after 2 months off work and revealed a PC_{20} value of 0.1 mg/mL.

Skin prick test results were positive with grass, tree, and weed pollens and negative for other perennial inhalants (fungi and dust mites). They were also positive with dog, cat, horse, cow, and pig dander, as well as with pork and lamb meat, and negative with rabbit, hamster, rat, and guinea pig dander. We prepared a pork bone extract in our laboratory using pork bone shavings from the cutting machine at the patient's workplace. The skin prick test with this extract was positive (7x6 mm).

Specific IgE (sIgE) was positive with dander from cat (26.9 kU_A/L), dog (>100 kU_A/L), horse (4.02 kU_A/L), cow (1.11 kU_A/L), guinea pig (1.87 kU_A/L), rat (0.43 kU_A/L), rabbit (1.74 kU_A/L), and pig (4.17 kU_A/L), as well as with pork (0.58 kU_A/L) and lamb (0.37 kU_A/L). Specific IgE to galactose-α-1,3-galactose was also measured, as it has been reported to be a cause of cross-reactivity between meat and dander. The result was negative (0.08 kU_A/L) [4].

An IgE dot blot was performed with pork bone, cat and dog dander, and other meat and albumin extracts. The result was positive for pork bone extracts, for beef and pork, and for pig, cat, and dog dander (Figure, A). A 12% SDS-PAGE and immunoblotting assay revealed IgE bands of different molecular weights. High-weight bands (>100 kDa) and medium-weight bands (57-67 kDa) were present in pork, cat, and dog extracts (Figure, B).

An immunoblot inhibition assay (Figure, C) revealed cross-reactivity between pork and cat or dog extracts; these were associated mainly with a medium-weight band. ImmunoCAP ISAC (Phadia) revealed species-specific allergens (Can f 1, 40 ISU-E; Can f 2, 11 ISU-E; Can f 5, 42 ISU-E; Fel d 1, 25 ISU-E; Fel d 4, 4.3 ISU-E) and cross-reactive allergens (Can f 3, 13 ISU-E; Fel d 2, 5 ISU-E).

Finally, the patient underwent a nasal challenge test with the pork bone extract to ascertain clinical relevance. The challenge was positive at 0.05 mg/mL. The patient developed intense rhinorrhea, sneezed 8 times, and experienced a 52% decrease in peak nasal inspiratory flow.

When the patient left her workplace, the severe symptoms resolved, leaving her with mild seasonal rhinoconjunctivitis only.

Pork-cat syndrome was described in 1994 in a report by Drouet et al [1], in which a patient who was allergic to cat dander developed exercise-dependent anaphylaxis after ingestion of pork. The authors found antigenic similarity between the serum albumin of both mammals [1]. This was the first report of cross-reactivity between the dander of one mammal and the meat of another.

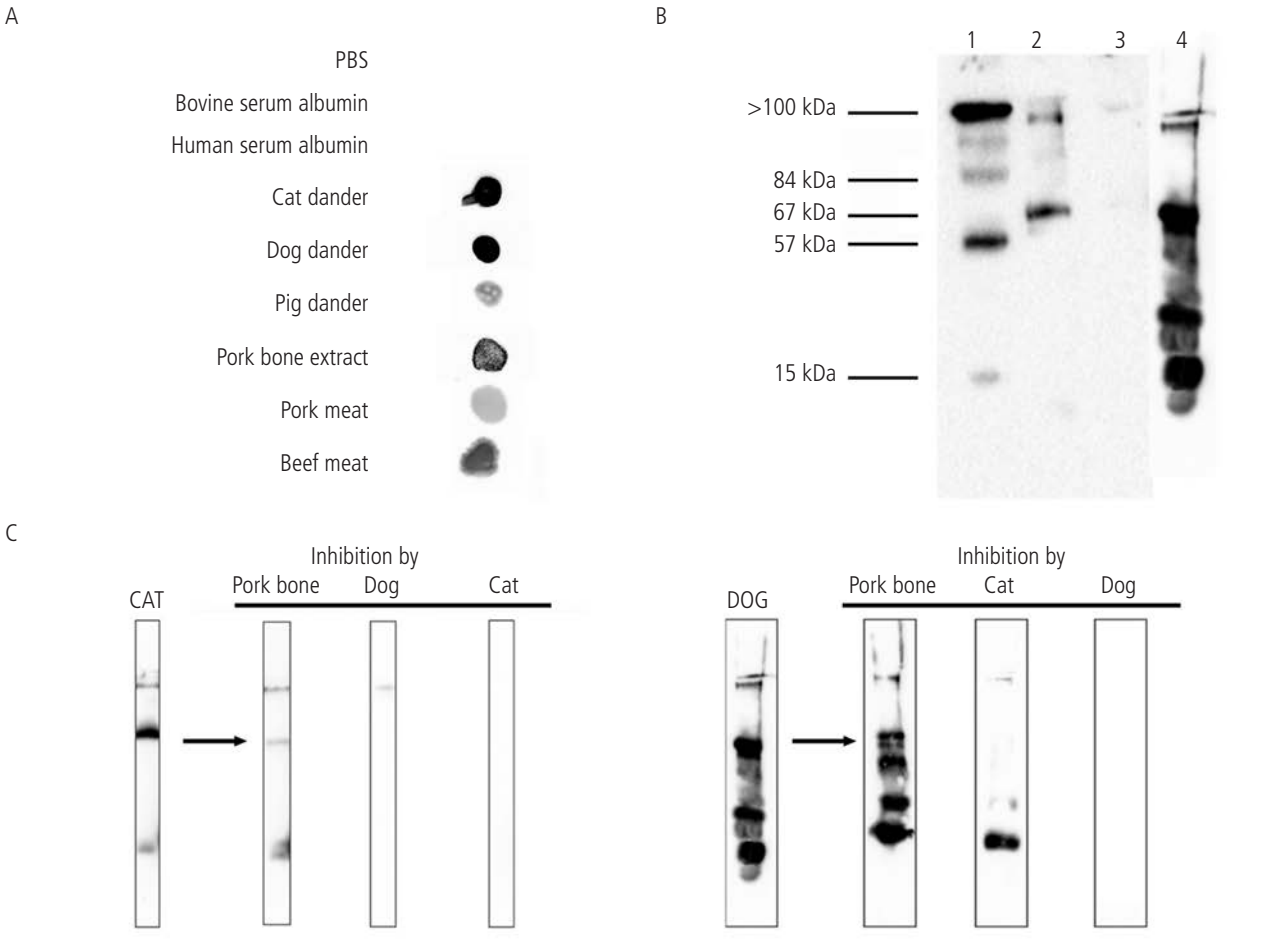

Figure. A, Detection of specific IgE to mammal dander, meat, albumins, and pork bone extracts (dot blot). B, Allergenic bands found in pork bone (1), cat dander (2), pork meat (3), and dog dander (4). C, Immunoblot inhibition. Left: Solid-phase: cat dander, inhibited by pork bone and dog and cat dander. Pork bone inhibits the 68-kDa band. Almost complete inhibition by dog. Right: Solid-phase: dog dander. Discreet inhibition of the 68-kDa band by pork bone. Cat dander inhibits more intensely. PBS indicates phosphate-buffered saline.

Although major structural homology can be identified between mammal albumins, wide variability in sensitization patterns has been reported [5-7]. The most common cross-reactions are between serum albumin from cat, dog, horse, and pork, as was the case in the patient we report [2].

Dog and cat serum albumin have been found to be cross-reactive in most patients who are allergic to mammal albumins. In addition, recombinant dog serum albumin has been proposed to be of use for the diagnosis of pork-cat syndrome and even for immunotherapy in patients who are allergic to mammal albumins [2].

In 1996, Drouet and Sabbah [3] studied the clinical relevance of this syndrome in 2 groups of atopic patients, depending on whether they had sIgE to pork or not. The authors found cat sensitization in 90% of pork-allergic patients, and only 45% in nonallergic patients [3]. These findings were corroborated by Higer et al [8].

Pork-cat syndrome has been described mainly in European patients. Nevertheless, the first series in the United States of America was recently reported by Posthumus et al [9].

Occupational asthma to pork has been increasingly documented. Of particular interest is the case of a pork industry worker who experienced respiratory symptoms with pork and later developed allergic reactions when eating chicken as a result of cross-reactivity with hemoglobin [10].

However, the case we report is of particular interest because it involves a patient who was allergic to cat and dog dander and sensitized to pork proteins (pork-cat syndrome). The patient's symptoms were caused by inhalation, and her disease was considered to be occupational. Sensitization to galactose-α-1,3-galactose, a source of cross-reactivity between meat and dander [4], was ruled out. Given the sequence of events, it seems highly probable that cat dander was the primary sensitizer.

In summary, we report an unusual case of occupational asthma resulting from pork-cat syndrome.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Previous Presentation

Data for this patient were presented in poster form at the XXVIII SEAIC congress, Pamplona, Spain, 2012.

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Manuscript received August 30, 2013; accepted for publication January 27, 2014.

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