

Letter to the Editor

Low prevalence of IgE to cross-reactive carbohydrate determinants in beekeepers

To the Editor:

N-glycans from plant and insect glycoproteins can induce extensive IgE cross-reactivity.^{1,2} IgE antibodies against these glycans, commonly known as cross-reactive carbohydrate determinants (CCDs), may interfere with *in vitro* tests for allergy diagnosis.² The cause of CCD sensitization is a matter of debate. *N*-glycans are ubiquitous in nature and represent the most common set of epitopes to which humans are exposed.² From the initial descriptions of CCDs, Hymenoptera stings have been proposed as a cause of CCD sensitization.¹ Serial determinations in patients with Hymenoptera venom allergy showed that CCD-specific IgE (sIgE) levels increased in parallel with the culprit venom-sIgE levels after a sting.³ In patients with suspected respiratory allergy, a history of Hymenoptera stings was associated with CCD-sIgE-positivity.⁴ The level of CCD-sIgE is higher in atopic than in nonatopic individuals^{4,5} and is highest among patients who are allergic to Hymenoptera venoms.⁶ For an in-depth understanding of the role of Hymenoptera venom exposure on CCD sensitization, we investigated the prevalence and factors associated with CCD-sIgE among beekeepers, a population that is heavily exposed to stings.

In this cross-sectional study, the 320 members of the Sociedad de Apicultores (Beekeeping Association) of Lugo (Spain) were invited to participate via a personal letter. A total of 158 individuals consented to participate in the study, which was approved by the Regional Ethics Committee. Three individuals who were already diagnosed as having Hymenoptera venom allergy undergoing specific immunotherapy were excluded. The study thus contained 155 individuals (125 males; median age, 57 years; range, 23-88 years). All participants completed a physician-administered structured questionnaire that included items related to residence, occupational activity, smoking, alcohol consumption, beekeeping activities, sting episodes, and reactions to stings. Reactions were classified as none, small local, large local (with a diameter >10 cm and peaking at 24-48 hours), or systemic. Skin prick tests to a standard panel of common inhalant allergens, including mites and pollens, were performed in all participants. Individuals with at least a positive reaction (>3 mm) were considered atopic.

Total serum IgE was assayed by using the Immulite-2000 system (Siemens, Gwynedd, United Kingdom). Allergen-sIgE was assayed by using the Immuno-CAP-250 system (Phadia, Uppsala, Sweden) and included sIgE to honeybee venom (HBV), yellow jacket venom (YJV), *Polistes dominulus* venom, natural (glycosylated) HBV phospholipase-A2, recombinant (nonglycosylated) HBV phospholipase-A2, and MUXF (the *N*-glycan from bromelain, a CCD marker, containing α -1,6-mannose (M), β -1,2-xylose (X), and core α -1,3-fucose (F) substitutions; UniCAP, Phadia).² Following the manufacturer's instructions, sIgE levels of 0.1 kU_A/L or more were considered as "positive" and corresponding individuals were considered to have IgE sensitization to CCDs. For some analyses and following traditional classifications, individuals with sIgE levels of 0.35 kU_A/L or more to both HBV and YJV

were considered to have double *in vitro* reactivity. We also assayed MUXF-specific IgG and HBV-specific IgG and IgG₄ by using the Immuno-CAP-250 system.

Only a minority of beekeepers demonstrated IgE sensitization to CCDs. A total of 12 (7.7%; 95% CI, 3.5-11.9) had CCD (MUXF)-sIgE levels of 0.1 kU_A/L or more and only 4 (2.6%) had levels of 0.35 kU_A/L or more. The maximum CCD-sIgE level was 2.21 kU_A/L. IgE sensitization to CCDs was positively associated with atopy and negatively associated with age (Table I). These associations were maintained after adjusting for confounders (data not shown). IgE sensitization to CCDs was independent of sex, smoking, alcohol consumption, duration of beekeeping, number of beehives, sting episodes, and time elapsed since the last sting. IgE sensitization to CCDs was positively associated with reactions' severity (Table I). The sum of large local reactions and systemic reactions was higher in beekeepers with IgE sensitization to CCDs than in those without it (41.7% vs 9.8%; $P = .006$). Three beekeepers were newly diagnosed with systemic reactions after Hymenoptera stings. IgE sensitization to CCDs was associated with higher levels of serum total IgE and venom-sIgE, as well as with double (HBV and YJV) reactivity in the whole group (Table I), as well as within the subgroup of atopic individuals (see Table E1 in this article's Online Repository at www.jacionline.org). A total of 102 individuals (65.8%) showed detectable levels of CCD-sIgG. Levels of CCD-sIgG were similar among individuals with and without IgE sensitization to CCDs. Likewise, IgE sensitization to CCDs was not significantly associated with HBV-sIgG or HBV-IgG₄ (Table I). Levels of CCD-sIgG were correlated with those of HBV-sIgG ($\rho = 0.429$; $P < .001$) and with those of HBV-sIgG₄ ($\rho = 0.164$; $P = .04$, Spearman's test).

The prevalence of IgE sensitization to CCDs in beekeepers was lower than that observed in different populations studied by the authors (see Table E2 in this article's Online Repository at www.jacionline.org). Specifically, the prevalence was lower than that observed in patients allergic to Hymenoptera venom⁶ and in alcoholic subjects⁵ from the same area (Galicia, Spain). In earlier studies, IgE sensitization to CCDs demonstrated a consistent association with alcohol consumption,⁵⁻⁸ which was not observed in this population. The prevalence of IgE sensitization to CCDs in beekeepers was similar or slightly lower than that observed in patients with suspected respiratory allergy⁴ from the area and in general adult populations from Spain,⁵ Portugal,⁷ and Denmark.⁸ However, the relatively low level of CCD-sIgE among beekeepers may not argue against a role for bee stings in CCD sensitization. Most probably, the high venom exposure after repeated stinging episodes could provoke a status of immunologic tolerance through the induction of regulatory T cells, as previously described.⁹ Notably, beekeepers also had the lowest levels of serum total IgE among the populations studied (Table E2). The association between hypersensitivity symptoms and CCD-sIgE could indicate that sensitization to the glycosidic epitope is a risk factor for the development of clinical allergy to the venom. Alternatively, IgE sensitization to CCDs could merely be an epiphenomenon that is more evident in individuals who are highly sensitized to the venom. Another possibility is that there is selection bias in the population because beekeepers with a tendency to

TABLE I. Demographic, epidemiologic, and immunologic characteristics of beekeepers with and without IgE sensitization to CCD*

	Beekeepers with CCD sensitization (n = 12)	Beekeepers without CCD sensitization (n = 143)	P value
Age (y)	49 (26 to 73)	58 (23 to 88)	.02
Sex (male)	10 (83.3)	115 (80.4)	.99
Smoking (current)	2 (16.7)	15 (10.5)	.80
Alcohol consumption (units/wk)	11 (0 to 42)	10 (0 to 109)	.91
Residence (rural)	7 (58.3)	77 (53.8)	.99
Occupation (farming)	1 (8.3)	27 (18.9)	.58
Beekeeping			
Duration (y)	18 (6 to 45)	20 (2 to 75)	.17
No. of beehives	18 (1 to 100)	8 (1 to 1000)	.08
Average no. of sting episodes per year	3 (2 to 60)	6 (1 to 300)	.26
No. of sting episodes in the past year	2 (0 to 60)	4 (0 to 300)	.12
Months since the last bee sting	8.3 (<1 to 34)	6.4 (<1 to 170)	.11
Grade of reaction to the last bee sting			
No reaction	2 (16.7)	65 (45.4)	
Small local reaction	5 (41.6)	64 (44.8)	
Extensive local reaction	3 (25.0)	13 (9.1)	<.001
Systemic reaction	2 (16.7)	1 (0.7)	
History of wasp stings (yes)	8 (66.7)	99 (69.2)	.99
Atopy (skin prick test positivity)†	7 (58.3)	27 (18.9)	.005
Total serum IgE (IU/mL)	171 (13 to 3117)	26 (<1 to 656)	<.001
IgE to HBV (kU _A /L)	4.72 (0.39 to >100)	0.52 (0.03 to 9.41)	<.001
IgE to natural HBV PLA2 (kU _A /L)	3.04 (0.19 to >100)	0.31 (0.00 to 4.59)	<.001
IgE to recombinant HBV PLA2 (kU _A /L)	0.68 (0.00 to 95.0)	0.10 (0.00 to 1.50)	.002
IgE to YJV (kU _A /L)	0.38 (0.06 to 4.60)	0.00 (0.00 to 2.79)	<.001
IgE to PDV (kU _A /L)	0.43 (0.05 to 3.77)	0.06 (0.03 to 1.35)	<.001
Double HBV and YJV IgE reactivity‡	6 (50.0)	6 (5.6)	<.001
IgG to MUXF (mg _A /L)	2.23 (<2 to 5.31)	2.52 (<2 to 33.3)	.29
IgG to HBV (mg _A /L)	8.84 (2.54 to 52.3)	11.8 (<2 to 135)	.30
IgG ₄ to HBV (mg _A /L)	2.09 (0.04 to >30)	3.23 (0.02 to >30)	.45

Data are medians and ranges (within parentheses) or absolute numbers and percentages (within parentheses). The χ^2 test (with Yate's correction, where appropriate) was used to compare proportions, and the Mann-Whitney test was used to compare numerical variables between groups.

PDV, *Polistes dominulus* venom; PLA2, phospholipase-A2.

*CCD sensitization was defined by a CCD-sIgE level of 0.1 kU_A/L or more to MUXF, N-glycan from bromelain.

†Positive skin prick tests to at least 1 of the inhalant allergens tested.

‡No. and percentage of individuals showing IgE level of 0.35 kU_A/L or more to both honeybee and wasp venom.

develop IgE reactivity and thus have reactions may be prone to leave this occupation.

It is known that both healthy and allergic subjects display allergen-specific T cells that recognize the same T-cell epitopes, so that the final response would depend on different number of exposures, doses, and additional subject characteristics.⁹ Studies on beekeepers offer the opportunity to investigate such differences. Future longitudinal studies in this population could elucidate the role of repeated stings in the balance between sensitization and tolerance to the venom and its glycosidic epitopes.

We thank the Asociación Provincial de Apicultores de Lugo (Lugo Beekeeping Society, APLA) for collaboration and Maria-José Gómez for technical assistance.

Francisco-Javier Carballada, MD^a
Arturo Gonzalez-Quintela, MD, PhD^b
Ramón Nuñez, MD^a
Carmen Vidal, MD, PhD^c
Manuel Boquete, MD, PhD^a

From ^athe Department of Allergy, Hospital Lucus Augusti, Lugo, Spain, and the Departments of ^bMedicine and ^cAllergy, Hospital Clínico Universitario, Santiago de Compostela, Spain. E-mail: arturo.gonzalez.quintela@usc.es.

This study was supported by the Sociedad Española de Alergia e Immunología Clínica (SEAIC) and the Instituto de Salud Carlos III (Spanish Ministry of Health, PI10/01870).

Disclosure of potential conflict of interest: All of the authors have received research support from the Spanish Society of Allergy and Clinical Immunology and the Spanish Ministry of Health.

REFERENCES

1. Aalberse RC, Koshte V, Clemens JG. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. *J Allergy Clin Immunol* 1981;68:356-64.
2. Altmann F. The role of protein glycosylation in allergy. *Int Arch Allergy Immunol* 2007;142:99-115.
3. Kochuyt AM, Van Hoeyveld EM, Stevens EA. Prevalence and clinical relevance of specific immunoglobulin E to pollen caused by sting-induced specific immunoglobulin E to cross-reacting carbohydrate determinants in Hymenoptera venoms. *Clin Exp Allergy* 2005;35:441-7.
4. Vidal C, Sanmartín C, Armisen M, Rodríguez V, Linneberg A, Gonzalez-Quintela A. Minor interference of cross-reactive carbohydrates with the diagnosis of respiratory allergy in standard clinical conditions. *Int Arch Allergy Immunol* 2012; in press. DOI: 10.1159/000324447.
5. Gonzalez-Quintela A, Garrido M, Gude F, Campos J, Linneberg A, Lojo S, et al. Sensitization to cross-reactive carbohydrate determinants in relation to alcohol consumption. *Clin Exp Allergy* 2008;38:152-60.
6. Carballada FJ, Gonzalez-Quintela A, Nuñez-Orjales R, Vizcaino L, Boquete M. Double (honeybee and wasp) immunoglobulin-E reactivity in patients allergic to Hymenoptera venom: the role of cross-reactive carbohydrates and alcohol consumption. *J Invest Allergol Clin Immunol* 2010;20:484-9.

7. Coutinho V, Vidal C, Garrido M, Gude F, Lojo S, Linneberg A, et al. Interference of cross-reactive carbohydrates in the determination of specific IgE in alcohol drinkers and strategies to minimize it: the example of latex. *Ann Allergy Asthma Immunol* 2008;101:394-401.
8. Linneberg A, Fenger RV, Husemoen LL, Vidal C, Vizcaino L, Gonzalez-Quintela A. Immunoglobulin E sensitization to cross-reactive carbohydrate determinants: epidemiological study of clinical relevance and role of alcohol consumption. *Int Arch Allergy Immunol* 2010;153:86-94.
9. Meiler F, Zumkehr J, Klunker S, Rückert B, Akdis CA, Akdis M. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. *J Exp Med* 2008;205:2887-98.
doi:10.1016/j.jaci.2011.08.002

TABLE E1. Demographic, epidemiologic, and immunologic characteristics of atopic beekeepers with and without IgE sensitization to CCD*

	Atopic beekeepers with CCD sensitization (n = 7)	Atopic beekeepers without CCD sensitization (n = 27)	P value
Age (y)	38 (26 to 55)	54 (31 to 82)	.03
Sex (male)	7 (100)	21 (77.8)	.30
Smoking (current)	1 (14.3)	2 (7.4)	.51
Alcohol consumption (units/wk)	15 (0 to 40)	8 (0 to 57)	.47
Residence (rural)	5 (71.4)	11 (40.7)	.21
Occupation (farming)	1 (14.3)	1 (3.7)	.37
Beekeeping			
Duration (y)	8 (6 to 40)	20 (2 to 70)	.11
No. of beehives	20 (1 to 100)	5 (1 to 200)	.15
Average no. of sting episodes per year	4 (2 to 50)	4 (1 to 100)	.64
No. of sting episodes in the past year	1 (0 to 50)	3 (0 to 100)	.50
Months since the last bee sting	8.6 (<1 to 33)	6.5 (<1 to 25)	.53
Reaction to the last bee sting (no reaction or small local reaction)	4 (57.1)	23 (85.2)	.13
History of wasp stings (yes)	5 (71.4)	20 (74.1)	.99
Total serum IgE (IU/mL)	178 (17 to 3117)	92 (<1 to 656)	.06
IgE to HBV (kU _A /L)	5.59 (0.39 to >100)	0.81 (0.03 to 9.06)	.01
IgE to natural HBV PLA2 (kU _A /L)	4.16 (0.19 to >100)	0.42 (0.00 to 4.59)	.008
IgE to recombinant HBV PLA2 (kU _A /L)	1.02 (0.00 to 95.0)	0.10 (0.00 to 1.50)	.06
IgE to YJV (kU _A /L)	1.39 (0.06 to 4.70)	0.03 (0.00 to 2.79)	.002
IgE to PDV (kU _A /L)	0.74 (0.07 to 3.77)	0.10 (0.05 to 0.45)	.006
Double HBV and YJV IgE reactivity†	5 (71.4)	4 (14.8)	.007
IgG to MUXF (mg _A /L)	2.35 (<2 to 5.31)	3.14 (0 to 12.6)	.28
IgG to HBV (mg _A /L)	16.4 (2.54 to 52.3)	11.3 (2.56 to 69.0)	.91
IgG ₄ to HBV (mg _A /L)	2.10 (0.04 to >30)	2.96 (0.02 to >30)	.78

Data are medians and ranges (within parentheses) or absolute numbers and percentages (within parentheses). Fisher's exact test was used to compare categorical variables and the Mann-Whitney test was used to compare numerical variables between groups.

PDV, *Polistes dominulus* venom; PLA2, phospholipase-A2.

*CCD sensitization was defined by a CCD-sIgE level of 0.1 kU_A/L or more to MUXF, N-glycan from bromelain.

†No. and percentage of individuals showing IgE level of 0.35 kU_A/L or more to both honeybee and wasp venom.

TABLE E2. Comparison of the prevalence of IgE to CCD in adult populations studied by the authors

Study population characteristics	Setting	Reference no.	No. of subjects	Age (y), median and (range)	Sex (male), %	Residence (rural), %	Atopy (skin prick test positivity), %	Heavy drinking (>28 units/wk), %	Smoking (current), %	Serum total IgE (IU/mL), median (range)	IgE to CCD (MUXF-sIgE level of ≥ 0.35 kU _A /L), % (95% CI)	IgE to CCD (MUXF-sIgE level of ≥ 0.10 kU _A /L), % (95% CI)
General adult population from a single municipality	A-Estrada, Galicia, Spain	[5]	457	54 (18 to 92)	44.0	100	26.0	9.6	21.7	59 (3 to 4312)	5.9 (3.7 to 8.1)	11.4 (8.5 to 14.3)
Adult patients attending an allergy clinic with respiratory symptoms	Santiago de Compostela, Galicia, Spain	[4]	1025	33 (18 to 79)	35.2	68.7	78.2	0.8	22.7	96 (2 to 6950)	4.3 (3.0 to 5.5)	12.1 (10.1 to 14.1)
Alcoholic subjects admitted to the hospital	Santiago de Compostela, Galicia, Spain	[5]	138	46 (27 to 78)	74.6	80.1	20.3	100	50.7	214 (1 to 4573)	18.8 (12.3 to 25.4)	34.1 (26.1 to 42.0)
Adult individuals attending a therapeutic spa resort	Xerés, Portugal	[7]	270	59 (27 to 83)	63.7	ND	ND	20.0	6.3	39 (1 to 2010)	1.8 (0.24 to 3.5)	7.4 (4.3 to 10.5)
General adult population from a single municipality	Copenhagen, Denmark	[8]	1197	38 (15 to 79)	45.8	0	38.9	5.0	36.8	37 (1 to 12,150)	2.1 (1.3 to 2.9)	6.4 (5.0 to 7.8)
Patients allergic to hymenoptera venom	Lugo, Galicia, Spain	[6]	88	44 (16 to 75)	72.5	79.1	15.4	22.0	29.7	98 (4 to 3209)	29.5 (20.0 to 39.1)	42.0 (31.7 to 52.4)
Nonallergic beekeepers*	Lugo, Galicia, Spain	Present study	152	57 (24 to 88)	80.9	54.6	21.7	19.7	11.2	27 (<1 to 3117)	2.6 (0.1 to 5.2)	6.6 (2.6 to 10.5)

All determinations were performed in the same laboratory.

CI, Confidence interval; ND, no data available.

*Patients with systemic reaction to Hymenoptera venom were excluded from this presentation.