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CORRESPONDENCE

Biologic activity of cross-reactive carbohydrate determinants in heavy drinkers

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We have read with interest the paper from Mertens et al. [1] reporting that cross-reactive carbohydrate determinants (CCDs) strongly affect the results of the basophil activation test in patients with hymenoptera venom allergy. We would like to extend this finding by showing that CCDs can even affect the results of basophil activation tests in non-allergic individuals with past hymenoptera exposure.

We performed a follow-up of three heavy drinkers who participated in an earlier study showing an increased prevalence of CCD-specific IgE in relation to alcohol consumption [2]. These patients (Table 1), all wine drinkers from rural areas, were originally admitted to the hospital due to alcohol withdrawal syndrome (Patients 1 and 2) or pneumonia (Patient 3) in 2002–2003 and had a high baseline concentration of CCD-specific IgE. At that time, they also showed high levels of IgE to pollens and hymenoptera venoms [2]. However, all had negative skin prick tests and no allergy symptoms. Preincubation of patients' sera with MUXF (the *N*-glycan from bromelain) conjugated to bovine serum albumin (BSA) induced a significant inhibition of IgE reactivity to pollens (Patients 1–3) and hymenoptera venoms (Patient 3). However, IgE reactivity to hymenoptera venoms was not significantly inhibited by preincubation with MUXF-BSA in Patients 1 and 2, suggesting that these patients might be also sensitized to hymenoptera venom proteins [2]. In fact, component-resolved diagnosis showed high levels of IgE to glycosylated venom allergens (particularly hyaluronidases) in all patients but also the presence of IgE to *Vespula* antigen 5 (a non-glycosylated allergen) in Patients 1 and 2 (Table 1). Five years later, in 2007–2008, these three patients were contacted for further studies after approval from the Institutional Review Board. One patient was abstinent and the other two had significantly reduced their alcohol intake (Table 1). They reported no respiratory allergy symptoms or reactions to foods, latex contact or insect stings. The three patients had all been stung by bees and/or wasps several times, with some stings occurring after the earlier hospital stay. Serum concentrations of total IgE and IgE specific to pollens, hymenoptera venoms, and venom components significantly decreased compared with IgE measurements during the previous admission. Reduction of IgE titres was higher than 10-fold in Patient 2, who was abstinent. The pattern of specific IgE inhibition after preincubation with MUXF-BSA remained similar. Of note, hymenoptera venoms and MUXF-BSA induced basophil activation in the three patients. Pollens induced basophil activation in Patients 2 and 3 (Table 1).

The source of CCD sensitization in the population is a matter of controversy. *N*-glycans are ubiquitous in nature, and exposure is common [4]. Hymenoptera stings have been considered a source of CCD sensitization because the initial description of CCDs in 1981 [5]. Data from these three patients could also indicate a role for hymenoptera stings, which combined with the enhancing effects of alcohol consumption on IgE synthesis [6] could promote CCD reactivity in alcoholics [7]. The dramatic decrease in both total and specific IgE levels after reduction of alcohol intake supports this hypothesis. In moderate and heavy drinkers, even a 2-week period of alcohol abstinence is followed by a significant decrease in serum levels of CCD-specific IgE [8].

The biological activity of CCDs is also a matter of controversy. Current thought is that CCDs have little activity *in vivo* [4, 9]. However, there are reports that CCDs have *in vitro* activity as evidenced by basophil activation in patients allergic to peanut [10], celery [11, 12], tomato [13, 14], olive pollen [15], cypress pollen [16], grass pollen [17], and hymenoptera venom [1]. Furthermore, the results presented support the ability of CCDs to induce basophil activation in asymptomatic individuals with past Hymenoptera exposure. Basophil activation by MUXF-BSA, pollens, and venoms has carbohydrates as the common denominator. This confirms recent reports indicate that allergologically asymptomatic, CCD-sensitized alcoholics exhibit positive basophil activation tests with a variety of CCD-bearing allergens [7]. Reasons for the discrepancies between *in vitro* and *in vivo* activity of CCDs are not clear [4, 18]. Concomitant IgG- or IgG_4-blocking antibodies, as detected in these three patients, could contribute to the *in vivo* irrelevance of CCDs [18]. The potential *in vitro* activity of CCDs in asymptomatic individuals should be taken into account when interpreting the results of basophil activation tests, at least among heavy drinkers.

Table 1. Demographic and clinical characteristics of patients

Patient	Patient 1 (#38)		Patient 2 (#85)		Patient 3 (#94)	
Gender/age (years)	M/56		M/51		M/52	
Hymenoptera stings (lifetime number)	> 10 (bees and wasps)		5–10 (bees and wasps)		> 10 (bees)	
Year of study	2002	2007	2003	2007	2003	2008
Alcohol consumption (g/day)	220	30	170	0	110	50
Allergy symptoms*	None	None	None	None	None	None
Skin prick tests†	Negative	Negative	Negative	Negative	Negative	Negative
Total IgE (IU/mL)	1840	643	819	80	232	136
Specific IgE (kU_A/L)‡						
MUXF	2.63	1.06	10.2	0.64	2.49	0.27
Olea europaea	3.12	1.30	12.9	0.81	3.52	0.55
Lolium perenne	4.14	1.55	17.7	1.06	3.61	0.63
HBV	74.3	36.1	65.2	6.16	5.35	1.15
YJV	5.87	1.77	14.9	1.31	2.67	0.57
Specific IgE to hymenoptera venom components (kU_A/L)§						
HBV phospolipase-A2 (Api m 1)	104.8	32.6	105.7	6.45	0.68	0.77
HBV hyaluronidase (Api m 2)	348.5	157.5	78.0	13.5	10.3	2.39
YJV phospholipase (Ves v 1)	0.18	0.31	8.8	1.64	1.98	0.99
YJV hyaluronidase (Ves v 2)	23.4	7.48	112.1	4.31	18.7	1.99
YJV antigen-5 (Ves v 5)	0.97	0	23.4	2.09	0.04	0
Inhibition of specific IgE with MUXF-BSA (%)¶						
O. europaea	Positive (85)	Positive (93)	Positive (86)	Positive (94)	Positive (80)	Positive (95)
L. perenne	Positive (68)	Positive (60)	Positive (66)	Positive (55)	Positive (73)	Positive (68)
HBV	Negative (15)	Negative (22)	Negative (17)	Negative (15)	Positive (52)	Positive (50)
YJV	Negative (22)	Positive (56)	Negative (48)	Negative (35)	Positive (74)	Positive (63)
Specific IgG/IgG_4 (mg/L)‡						
O. europaea	ND	11.7/< 0.15	ND	13.2/< 0.15	ND	4.0/< 0.15
L. perenne	ND	5.6/< 0.15	ND	10.4/0.18	ND	2.4/0.22
HBV	ND	5.6/0.30	ND	13.0/0.49	ND	3.7/0.88
YJV	ND	4.8/< 0.15	ND	9.5/< 0.15	ND	2.2/0.15
Basophil activation (%)‖						
Basal response	ND	19	ND	3	ND	6
Anti-IgE	ND	94	ND	89	ND	77
MUXF-BSA	ND	Positive (40)	ND	Positive (78)	ND	Positive (84)
O. europaea	ND	Negative (17)	ND	Positive (45)	ND	Positive (76)
L. perenne	ND	Negative (21)	ND	Negative (11)	ND	Positive (49)
HBV	ND	Positive (89)	ND	Positive (84)	ND	Positive (31)
YJV	ND	Negative (26)	ND	Positive (82)	ND	Positive (30)

*Systemic reaction to hymenoptera sting, or nasal, bronchial or skin disease.

†Skin prick tests to pollens, mites, pets, moulds, latex, and peanut.

‡UniCAP (Phadia, Sweden).

§ADVIA-Centaur (Siemens Medical Solutions Diagnostics, Munich, Germany). Allergens (ALK-Abello, Madrid, Spain) are natural with the exception of YJV phospholipase (Ves v 1), which was obtained by recombination in a Baculovirus system, and therefore is glycosylated.

¶UniCAP-inhibition assays. Figures represent the percentage of inhibition of specific IgE results after incubation of serum with MUXF-BSA (100 μg/mL), as described previously [2]. Criterion for positivity was extensive (⩾ 50%) inhibition.

‖Flow cytometric cellular allergen stimulation test based on CD63 expression, as previously described [3]. Figures represent the maximum percentage of activated basophils after challenge with allergens, including *O. europaea* (50 and 12.5 μg/mL; ALK-Abelló), *L. perenne* (80 and 20 μg/mL; ALK-Abelló), honeybee venom (5, 0.5, and 0.05 μg/mL; ALK-Abelló), yellow jacket venom (5, 0.5, and 0.05 μg/mL; ALK-Abelló), and MUXF-BSA (50, 5, and 0.5 μg/mL; Institute of Chemistry, Universitaet fuer Bodenkultur, Vienna, Austria). Criteria for positivity were (a) activation > 15% of cells and (b) activation > 2 times the baseline.
MUXF, the *N*-glycan of bromelain; BSA, bovine serum albumin; HBV, honeybee venom; YJV, yellow jacket venom; ND, not determined.

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