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Sensitization to cross-reactive carbohydrate determinants in relation to alcohol consumption

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Clinical and
Experimental
Allergy

Summary

Background Alcohol consumption is associated with increased serum IgE of unknown specificity.

Objective To investigate the prevalence of specific IgE to cross-reactive carbohydrate determinants (CCDs) in adults, and its relation to alcohol consumption.

Methods Population-based survey of 457 adults (218 abstainers, 195 light-to-moderate drinkers, 44 heavy drinkers). Specific IgE determinations included a CCD (MUXF³, the N-glycan of bromelain), pollens (*Lolium perenne* and *Olea europaea*), Hymenoptera venoms (*Apis mellifera* and *Vespula* spp.), and a mite (*Dermatophagoides pteronyssinus*).

We replicated these studies in an additional sample of alcoholics ($n = 138$). Inhibition assays were performed in selected cases.

Results In the general population, 5.6% of individuals (95% confidence interval 3.5–7.6%) showed positive (≥ 0.35 kU/L) CCD-specific IgE. The levels of CCD-specific IgE were particularly high in heavy drinkers, who also showed a high prevalence of positive IgE to pollens and Hymenoptera venoms, doubling (at least) the prevalence found in alcohol abstainers and light-to-moderate drinkers. The presence of IgE to pollens and Hymenoptera venoms was closely correlated with the presence of CCD-specific IgE. These features were confirmed in the additional sample of alcoholics. Inhibition studies indicated a role of CCD interference in IgE positivity to pollen and Hymenoptera allergens in alcoholics.

Conclusions CCD-specific IgE is prevalent in heavy drinkers, and is associated with positive IgE to pollens and Hymenoptera venoms. Specific IgE results should be interpreted with caution in heavy drinkers.

Keywords alcohol, cross-reactive carbohydrate determinants, ethanol, glycan, grass pollen, Hymenoptera, IgE, olive pollen

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Introduction

Alcohol consumption is consistently associated with increased serum concentrations of total IgE. Since initial descriptions [1], several studies have confirmed the finding of high serum IgE in alcoholics [2–4]. Moreover, moderate-to-heavy alcohol drinkers show an increase in total serum IgE when compared with abstainers [5–7]. The association of alcohol consumption with increased IgE levels is independent of age, sex, liver disease, nutrition, and smoking [1–7]. Furthermore, alcohol consumption during pregnancy is associated with increased cord blood

IgE concentrations [8]. In rodents, alcohol administration is shortly followed by an increase in serum IgE [9]. The mechanisms for IgE elevation in alcohol consumers are not entirely known. Alcohol is a powerful immunomodulatory drug that can intervene in almost every aspect of the immune response, either directly or indirectly by enhancing gut absorption of endotoxin [10, 11].

The association of alcohol consumption with allergic sensitization as evaluated by skin prick tests (SPTs) or specific IgE (sIgE) is controversial [6, 7, 12, 13]. Alcohol consumption is associated with positivity of multi-allergen sIgE tests that are used for allergy screening [11, 14].

Moreover, the proportion of individuals with positive multi-allergen sIgE tests and discordant-negative SPT is higher in heavy drinkers than in abstainers or moderate drinkers [11]. This may be an indication of the presence of sIgE to cross-reactive carbohydrate determinants (CCDs) in heavy drinkers. CCDs are oligosaccharide side chains *N*-linked to asparagine residues of plant and animal glycoproteins that are gaining attention as highly cross-reactive IgE-binding structures [15–17]. Current thought is that IgE antibodies to CCDs display poor biological activity because they are associated with neither SPT positivity nor clinical symptoms in the majority of cases [15, 16, 18]. However, CCD-sIgE can interfere with *in vitro* determinations by inducing sIgE positivity to plant and Hymenoptera venom allergens [15–17]. The risk of CCD interference may be higher when heterogeneous allergens are mixed in the same reagent [17], as in multi-allergen sIgE tests. Therefore, we investigated the prevalence of CCD-sIgE in relation to alcohol consumption.

Methods

Study populations

The study used two surveys aiming to investigate immunological alterations associated with alcohol consumption, as detailed elsewhere [7, 19]. The study was primarily focused on the first survey, which included an age-stratified random sample of 469 adult individuals of the municipality of A-Estrada, in north-western Spain [7]. Serum samples were available for 457 individuals. Their median age was 54 years (range 18–92 years). A total of 201 (44.0%) were males. According to their habitual alcohol consumption, 218 individuals (47.7%) were classified as alcohol abstainers, 195 (42.7%) as light-to-moderate drinkers, and 44 (9.6%) were classified as heavy drinkers. The proportion of heavy drinkers was similar to that observed in previous surveys in the same area [20].

The second survey consisted of 138 alcoholics consecutively admitted to the reference hospital [19]. Their median age was 47 years (range 27–78 years). A total of 103 (76.4%) were males. This population of alcoholics was considered separately in order to replicate the findings observed in heavy drinkers from the general adult population.

Alcohol consumption

Habitual alcohol intake was evaluated by the system of standard drinking units that sums the number of glasses of wine (~10 g), beers (~10 g), and units of spirits (~10 g) consumed per week, as described previously [7, 11, 19]. For this study, individuals with only occasional alcohol consumption were included among abstainers. Individuals who consumed 10–280 g/week were grouped as

light-to-moderate drinkers and those who consumed >280 g/week were considered heavy drinkers.

Specific immunoglobulin E measurements

Serum samples were stored frozen until tested in a UniCAP 250 analyser (Phadia, Uppsala, Sweden). The lower detection threshold was 0.01 kU/L. sIgE levels ≥ 0.01 were considered to be 'detectable'. Following traditional classifications, sIgE levels ≥ 0.35 kU/L ('class 1') were considered to be 'positive', and sIgE levels ≥ 3.5 kU/L ('class 3') were considered to be 'high'. We measured sIgE to the following commercially available allergens (Phadia):

- MUXF³ (Ro214), the *N*-glycan epitope from bromelain. This CCD, like all known *N*-glycans, has a backbone with two *N*-acetylglucosamines and a mannose. 'M' indicates the additional presence of a terminal α -mannose, 'U' indicates an unsubstituted 3-hydroxyl on the β -mannose, 'X' indicates a xylose, and 'F³' indicates the presence of a core α 1,3-fucose [15, 17]. Xylosilation and fucosylation are crucial for the antigenic properties of CCDs [15, 21, 22].
- *Lolium perenne* (ryegrass pollen, g5). Grass pollens are common in the area and a frequent cause of respiratory allergy [23, 24]. Glycans from grass pollen glycoproteins may contain both xylose and core α 1,3-fucose [15, 21].
- *Olea europaea* (olive tree pollen, t9). Olive tree pollen is both uncommon [23] and a very rare cause of respiratory allergy in the area. Glycans from olive pollen glycoproteins may contain both xylose and core α 1,3-fucose [25].
- *Apis mellifera* (honeybee) and *Vespula* spp. (yellow jacket) venoms (i1 and i3, respectively). These Hymenoptera are present in the area but are relatively rare causes of systemic anaphylaxis. Glycans from Hymenoptera venom glycoproteins contain core α 1,3-fucose but not xylose [26–28].
- *Dermatophagoides pteronyssinus* (house dust mite, d1). Mites are very common and the most frequent cause of respiratory allergy in the area [24]. Glycans from mite glycoproteins are not core 3-fucosylated [22].

Additional evaluations

All individuals underwent a panel of SPTs to relevant aeroallergens that included mites, pollens, moulds, and pets (dog and cat), as described previously [11, 24]. Individuals with at least one positive SPT to any of these allergens were considered to be SPT positive. SPT results and respiratory symptoms in these populations have been reported elsewhere [11, 24]. Only two individuals from the general population reported a diagnosis of Hymenoptera stinging hypersensitivity.

CAP-inhibition assays

Inhibition of sIgE results by CCD was investigated in selected alcoholics belonging to any of the following groups: (a) SPT-negative individuals with positive CCD-sIgE ($n = 5$), (b) SPT-positive individuals with positive CCD-sIgE ($n = 5$), and (c) SPT-positive individuals with undetectable or low-level CCD-sIgE ($n = 5$). The solid phase included *L. perenne*, *O. europaea*, *A. mellifera*, *Vespula* spp., or *D. pteronyssinus* depending on the sensitization profile in each case. The inhibitor was a neoglycoprotein (MUXF³-BSA) consisting of bromelain glycopeptides with an MUXF³ structure conjugated to bovine serum albumin (BSA) [22]. The conjugate, which contains three to four MUXF³-type glycans per molecule of BSA, was obtained from the Institute of Chemistry, Universitaet fuer Bodenkultur, Vienna, Austria. For inhibition assays, 150 µL of serum was pre-incubated with 150 µL of the PBS-buffered MUXF³-BSA solution to give a final conjugate concentration of 100 µg/mL. This concentration was chosen after performing CAP inhibition dose-response curves with increasing concentrations (4, 20, and 100 µg/mL) in some patients, as described previously [29]. After overnight coincubation at 4 °C, CAP results were assessed as usual. BSA was run as negative control in every case.

Statistical analyses

The χ^2 test, the Mann-Whitney test, and the Spearman rank test were used where appropriate. Logistic regression was used to adjust for confounding. All potential confounders were forced to enter the equation. Alcohol consumption was introduced in categories, using abstainers as the reference. For calculations of overall prevalence referred to the general population, compensatory weights were developed to obtain estimates taking into account the age-stratified sampling. Two-tailed P -values < 0.05 were considered to be statistically significant.

Ethical issues

All individuals consented to participate in the study, which was reviewed and approved by the regional Ethics Committee.

Results

Cross-reactive carbohydrate determinant – specific immunoglobulin E – is detectable in a high proportion of the general adult population

Serum levels of sIgE to CCD (MUXF³) in the general adult population ranged from undetectable (< 0.01 kU/L) to 7.09 kU/L. A total of 115 individuals [25.2%, weighted proportion 25.7%, 95% confidence interval (CI) 21.6–29.7%] showed detectable (≥ 0.01 kU/L) CCD-sIgE

levels. Of these, 27 individuals (5.9%, weighted proportion 5.6%, 95% CI 3.5–7.6%) showed serum CCD-sIgE levels traditionally considered 'positive' (≥ 0.35 kU/L). CCD-sIgE positivity was not significantly associated with respiratory symptoms (data not shown).

Cross-reactive carbohydrate determinant – specific immunoglobulin E levels are increased in heavy drinkers

In the general population, the highest serum levels of CCD-sIgE were found in heavy drinkers (Fig. 1). This finding was confirmed among in-hospital alcoholics (Fig. 1). In the general population, the association between heavy drinking and CCD-sIgE positivity (≥ 0.35 kU/L) was still present after adjusting for age, sex, smoking, and SPT positivity [adjusted odds ratio (OR) 3.56, 95% CI 1.09–11.6, $P = 0.03$]. In the same model, smoking was not significantly associated with CCD-sIgE positivity (adjusted OR 1.17, 95% CI 0.43–3.15, $P = 0.75$).

Specific immunoglobulin E levels to pollen and Hymenoptera venom allergens bearing cross-reactive carbohydrate determinants are increased in heavy drinkers

Heavy drinkers from the general population showed significantly higher levels of sIgE to pollens (*L. perenne* and *O. europaea*) and Hymenoptera venoms (*A. mellifera* and *Vespula* spp.) than abstainers and moderate drinkers (Fig. 1). After adjusting for age, sex, smoking, and SPT positivity, heavy drinking was associated with sIgE positivity (≥ 0.35 kU/L) to *L. perenne* (OR 4.8, 95% CI 1.7–13.7, $P = 0.004$), *O. europaea* (OR 3.1, 95% CI 1.1–9.2, $P = 0.03$), *A. mellifera* (OR 6.7, 95% CI 3.0–15.0, $P < 0.001$), and *Vespula* spp. (OR 3.4, 95% CI 1.5–7.7, $P = 0.004$). This finding was confirmed among in-hospital alcoholics (Fig. 1).

There was a strong correlation between CCD-sIgE levels and sIgE levels to *L. perenne*, *O. europaea*, *A. mellifera*, and *Vespula* spp., particularly among SPT-negative individuals (Fig. 2). The correlation between CCD-sIgE and sIgE to venoms was somewhat lower than that observed between CCD-sIgE and sIgE to pollens. All but one out of the 27 individuals (96.3%) with positive (≥ 0.35 kU/L) CCD-sIgE levels also showed positive sIgE to *L. perenne*, *O. europaea*, and *A. mellifera*, and all but two (92.6%) also presented positive sIgE to *Vespula* spp. The proportion of individuals with positive sIgE to both venoms was 10.6% in abstainers, 9.2% in light-to-moderate drinkers, and 34.1% in heavy drinkers ($P < 0.001$). Individuals with positive sIgE to both venoms showed the highest levels of CCD-sIgE (Fig. 3). These findings were confirmed among in-hospital alcoholics (Figs 1 and 2). Seven alcoholics (5.0%) presented titres of sIgE ≥ 35 kU/L to either *A. mellifera* or *Vespula* spp.

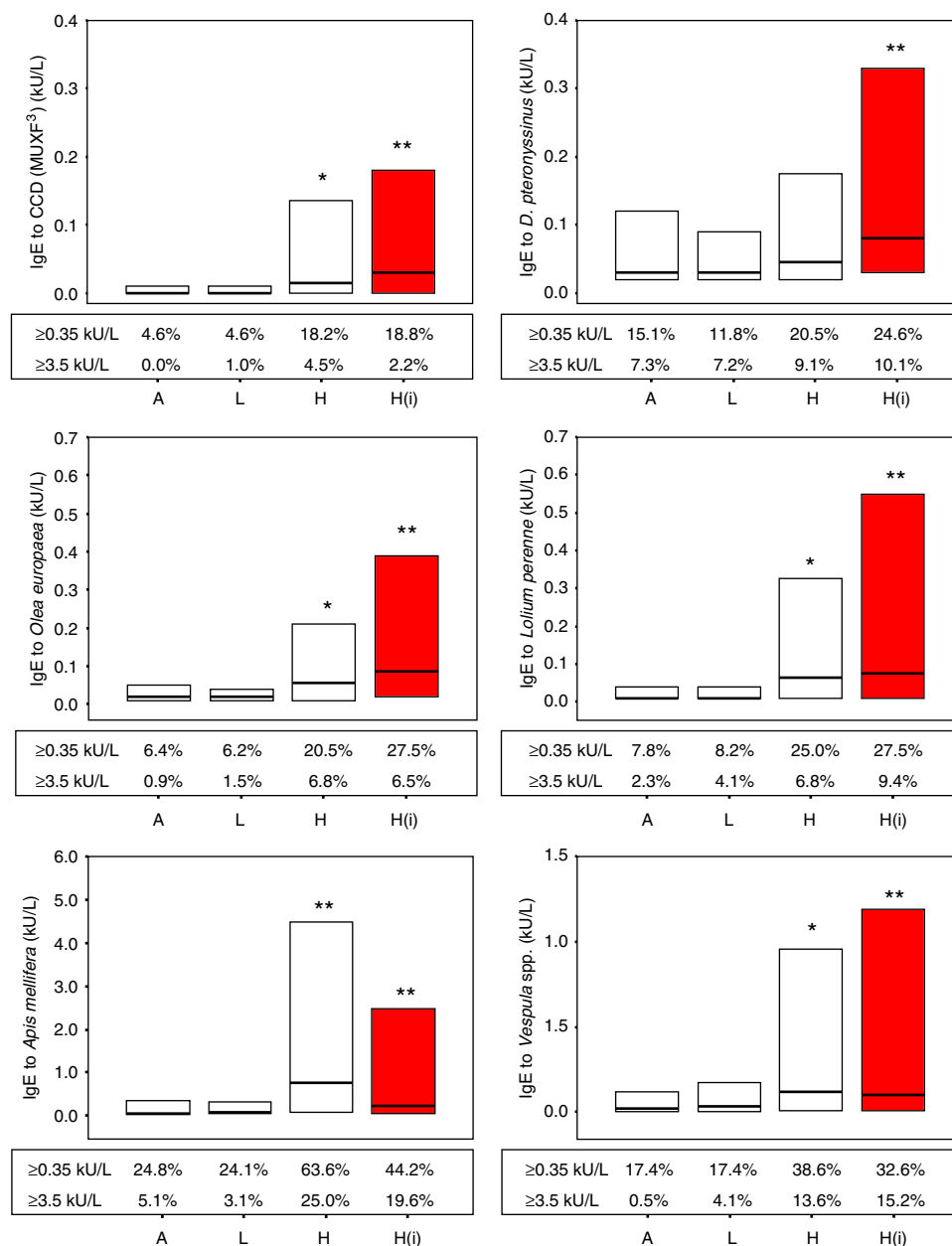


Fig. 1. Levels of sIgE against CCD (MUXF³) and the studied allergens in relation to alcohol consumption. Boxes represent interquartile ranges and median values. Numbers represent the percentage of individuals with sIgE ≥ 0.35 kU/L (class ≥ 1) and ≥ 3.5 kU/L (class ≥ 3). A, abstainers ($n = 218$); L, light-to-moderate drinkers ($n = 195$); H, heavy drinkers ($n = 44$); H(i), in-hospital alcoholics, which are further identified as red boxes ($n = 138$). * $P < 0.005$, ** $P < 0.001$ (respect to abstainers and to light-to-moderate drinkers). CCD, cross-reactive carbohydrate determinant; sIgE, specific IgE; *D. pteronyssinus*, *Dermatophagoides pteronyssinus*.

Levels of sIgE to *D. pteronyssinus* tended to be higher in heavy drinkers than in alcohol abstainers or moderate drinkers from the general adult population, although the difference was not significant (Fig. 1). There was some correlation between CCD-sIgE and sIgE to *D. pteronyssinus*, but lower than that observed for pollens and Hymenoptera venoms (Fig. 2). Only 12 out of 27 individuals (44.4%) with positive CCD-sIgE showed positive IgE to *D. pteronyssinus*.

Discordance between specific immunoglobulin E and skin prick test to some allergens is more frequent among heavy drinkers, and is related to the presence of cross-reactive carbohydrate determinant – specific immunoglobulin E

Discordant positive results of sIgE [i.e. presence of positive (≥ 0.35 kU/L) sIgE to a given allergen with negative SPT to the same allergen] were more common for *L. perenne* than for *D. pteronyssinus* (Fig. 4). In the general

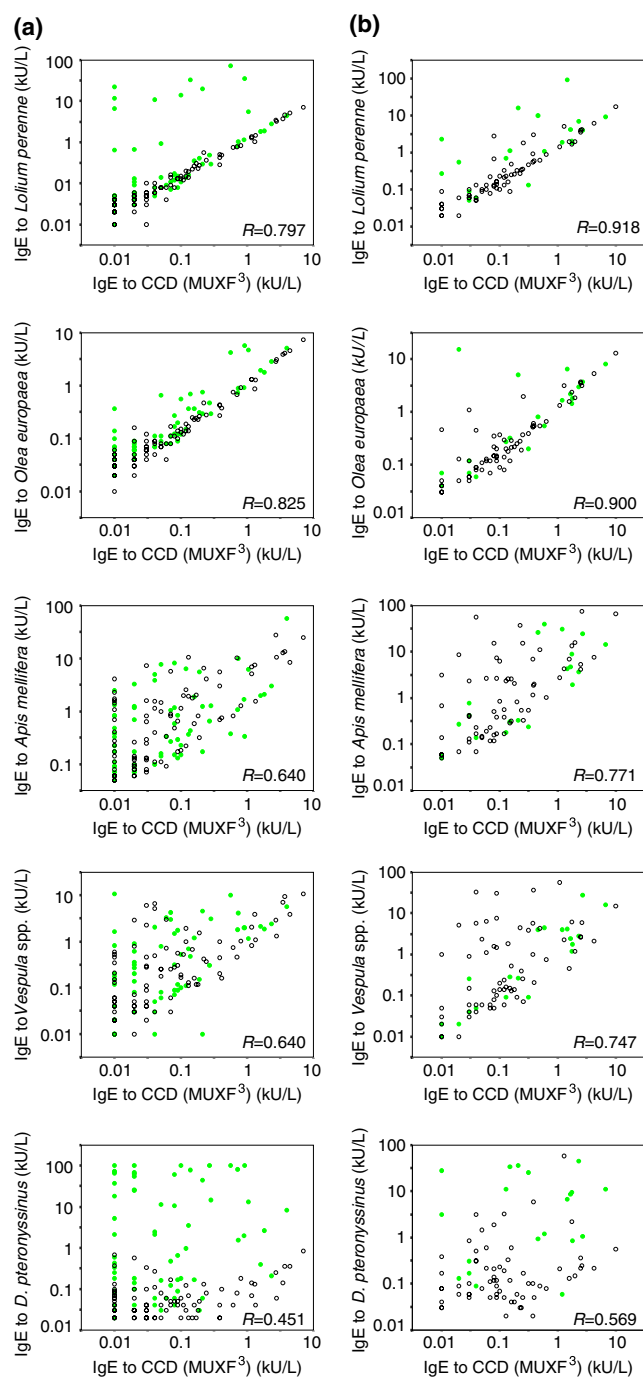


Fig. 2. Scatterplot of sIgE to allergens in relation to sIgE against CCD (MUXF³). (a) General population (left panels); (b) in-hospital alcoholics (right panels). Green dots and empty dots represent SPT-positive and SPT-negative individuals, respectively. Only cases with detectable sIgE are represented. Calculation of correlation coefficients (R) included all cases. All coefficients are statistically significant. CCD, cross-reactive carbohydrate determinant; sIgE, specific IgE; *D. pteronyssinus*, *Dermaphagoides pteronyssinus*.

population, the proportion of individuals with discordant positive sIgE to *L. perenne* was higher in heavy drinkers than in alcohol abstainers or light-to-moderate drinkers (Fig. 4). This finding was confirmed among in-hospital

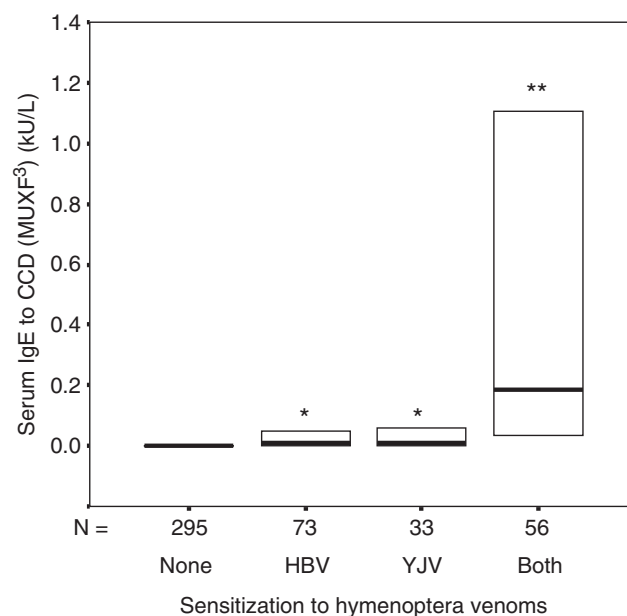


Fig. 3. Levels of sIgE to CCD (MUXF³) in individuals from the general population, classified according to the results of sIgE against Hymenoptera venoms. HBV, individuals with honey bee (*Apis mellifera*) venom positivity (≥ 0.35 kU/L); YJV, individuals with yellow jacket (*Vespula* spp.) venom positivity; Both, individuals with double positivity; None, individuals with no sensitization (sIgE to both venoms < 0.35 kU/L). Boxes represent interquartile ranges and median values. * $P < 0.001$ with respect to individuals with no sensitization; ** $P < 0.001$ with respect to every other group. CCD, cross-reactive carbohydrate determinant; sIgE, specific IgE.

alcoholics (Fig. 4). Among individuals with positive sIgE to *L. perenne*, CCD-sIgE levels were higher in those with negative SPT than in those with positive SPT (data not shown).

Discordant positive sIgE results to *D. pteronyssinus* were not so clearly associated with alcohol consumption in the general adult population (Fig. 4). Among individuals with a positive (≥ 0.35 kU/L) sIgE to *D. pteronyssinus*, CCD-sIgE levels were similar in individuals with negative SPT to this mite and in those with a positive SPT (data not shown). The proportion of discordant positive sIgE to *D. pteronyssinus* was higher in alcoholics admitted to the hospital (Fig. 4). In these cases, however, discordance was not associated with CCD-sIgE (data not shown).

CAP-inhibition studies indicate that specific immunoglobulin E positivity to some allergens in alcoholics is due to cross-reactivity with cross-reactive carbohydrate determinant

The individual results of CAP-inhibition assays in representative alcoholics are shown in Table 1. In alcoholics with positive CCD-sIgE ($n = 10$), MUXF³-BSA showed extensive ($\geq 50\%$) inhibition of sIgE to *L. perenne* in seven cases (inhibition range 59–88%), to *O. europaea* in

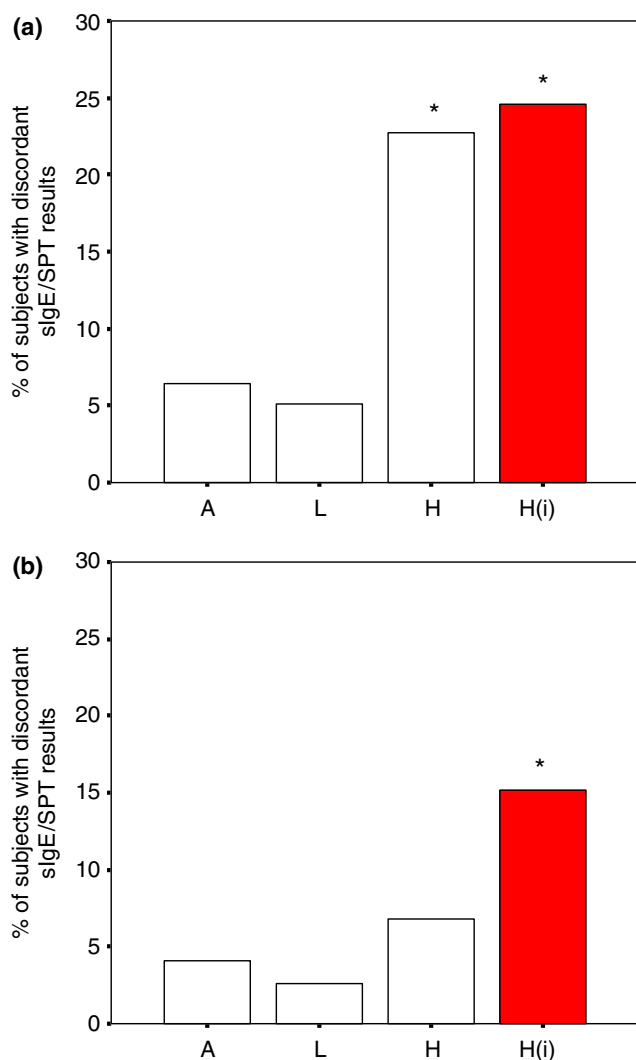


Fig. 4. Proportion of individuals with discordant positive sIgE/SPT results (sIgE ≥ 0.35 kU/L with negative SPT to the same allergen) in relation to alcohol consumption. (a) Discordant sIgE/SPT to *Lolium perenne*. (b) Discordant sIgE/SPT to *Dermatophagoides pteronyssinus*. A, abstainers ($n = 218$); L, light-to-moderate drinkers ($n = 195$); H, heavy drinkers ($n = 44$); H(i), in-hospital alcoholics, which are further identified as red bars ($n = 138$). * $P < 0.001$ with respect to abstainers and to light-to-moderate drinkers. SPT, skin prick test; sIgE, specific IgE.

eight cases (inhibition range 63–93%), to *A. mellifera* in four cases (inhibition range 50–75%), and to *Vespula* spp. in six cases (inhibition range 50–87%). Inhibition in CCD-sIgE-positive alcoholics was similar in cases with and without positive SPT. In alcoholics with negative CCD-sIgE and positive SPT to pollens, MUXF³-BSA induced no inhibition of sIgE to these pollens (inhibition $<15\%$ in all cases). MUXF³-BSA induced no inhibition of sIgE to *D. pteronyssinus*, either in cases with or without CCD-sIgE (inhibition $<15\%$ in all cases). The negative control (BSA) showed no inhibition in any case.

Discussion

The existence of sIgE to glycans was reported more than 25 years ago [30]. Subsequent reports have emphasized the interference of glycans (CCDs) in sIgE determinations due to widespread cross-reactivity [15, 31, 32]. Mari [32] reported a 23% prevalence of CCD-sIgE positivity among 1831 subjects with suspicion of respiratory allergy but, to our knowledge, no study has focused on the prevalence of CCD-sIgE in a general population. This study shows that a sizeable proportion of adults have detectable levels of IgE to a CCD that contains xylose and core $\alpha(1,3)$ -fucose (MUXF³). Titres are generally low, but are accompanied by sIgE positivity to plant and Hymenoptera venom allergens. Furthermore, we observed that the prevalence of CCD-sIgE is higher in heavy drinkers than in alcohol abstainers or light-to-moderate drinkers. Heavy drinkers also show a high prevalence of sIgE to plant and Hymenoptera allergens, probably due to CCD interference.

Positivity of sIgE to *L. perenne* was about three times more frequent in heavy drinkers than in abstainers or light-to-moderate drinkers. It seems likely that much of this association is due to CCD interference because (a) the vast majority of positive cases seemed 'false' (i.e. were discordant with SPT results), (b) discordance between sIgE and SPT to this pollen was associated with high titres of CCD-sIgE, (c) levels of sIgE to the CCD were strongly correlated with those of sIgE to pollen glycoprotein, which may bear identical glycans [16], (d) correlation was particularly strong in non-atopic subjects, and (e) IgE reactivity to *L. perenne* was extensively inhibited by pre-incubation with MUXF³-BSA in these cases. Similarly, heavy drinkers showed an exceedingly high rate of sIgE to *O. europaea*, which, of note, is rare in the area. Because of its rarity, olive pollen was not included in the panel of SPT when the source study was planned. Therefore, we cannot affirm that these olive-positive cases are 'false', but CCD interference seems likely because (a) positive cases were strongly correlated with CCD-sIgE positivity and (b) selected cases were also inhibited by pre-incubation with MUXF³-BSA. These results are not unexpected, because glycans from *O. europaea* glycoproteins also contain xylose and core $\alpha(1,3)$ -fucose [25]. Consequently, it seems likely that CCD-sIgE could interfere with the determination of sIgE to additional plant glycoproteins containing similar glycan structures [15, 16].

The prevalence of sIgE to *A. mellifera* or *Vespula* spp. was particularly high among heavy drinkers. More than a half of the heavy drinkers showed sIgE positivity to *A. mellifera* and more than a third to *Vespula* spp. This finding may be of importance because (a) the diagnostic value of sIgE is specially relevant for allergies having life-threatening potential [15], (b) SPT to Hymenoptera venoms are frequently negative in cases of true allergy, the diagnosis often relying on *in vitro* tests [33], and (c) some

Table 1. Demographic data, specific immunoglobulin E results, and results of CAP inhibition with a conjugate of cross-reactive carbohydrate determinant linked to bovine serum albumin (MUXF³-BSA) in 15 representative alcoholics

Group	Patient no.	Age (years)	Sex	Positive SPT	sIgE (kU/L)						CAP inhibition with MUXF ³ -BSA (%)				
					MUXF ³ (CCD)	OE	LP	HBV	YJV	DP	OE	LP	HBV	YJV	DP
A	38	50	M	None	2.63	3.12	4.14	74.30	5.87	0.25	85	68	15	22	ND
A	77	52	M	None	4.31	5.32	6.11	7.62	2.10	0.22	87	83	74	86	ND
A	85	47	M	None	10.2	12.9	17.7	65.2	14.9	0.57	86	66	17	48	ND
A	94	47	M	None	2.49	3.52	3.61	5.35	2.67	0.36	80	73	52	74	ND
A	99	58	M	None	2.53	3.70	4.01	4.23	2.61	0.22	33	24	43	87	ND
B	14	39	M	Pollens	1.76	1.44	2.01	8.95	1.19	9.64	93	64	8	75	0
B	81	52	M	Pollens, mites	2.33	3.02	6.97	3.67	2.78	44.9	89	37	75	70	0
B	128	37	M	Mites	2.70	3.72	4.09	24.5	27.8	1.07	88	88	17	10	13
B	131	39	M	Pollens, mites	1.48	6.43	94.1	4.27	4.20	6.83	38	5	50	45	0
B	135	41	M	Mites	6.72	8.01	9.14	14.4	16.0	11.1	63	59	46	50	5
C	4	55	M	Mites	0.15	0.32	1.14	0.31	0.29	33.7	ND	13	ND	ND	0
C	8	33	M	Mites	0.21	5.04	16.3	0.33	0.27	36.0	9	0	ND	ND	0
C	9	38	M	Pollens, mites	0.01	0.07	2.30	0.05	0.01	3.10	ND	0	ND	ND	0
C	92	58	F	Pollens	0.02	15.4	0.56	0.27	0.02	0.13	8	ND	ND	ND	ND
C	98	36	M	Mites	0.00	0.43	0.81	0.06	0.02	8.14	ND	ND	ND	ND	6

Group A: Skin prick test-negative individuals with positive (≥ 0.35 kU/L) CCD-sIgE.

Group B: Skin prick test-positive individuals with positive (≥ 0.35 kU/L) CCD-sIgE.

Group C: Skin prick test-positive individuals with undetectable or low-level CCD-sIgE.

Figures for CAP inhibition indicate the maximum percentage inhibition obtained with 100 µg/mL inhibitor protein. Percentages of inhibition have been approximated to the nearest whole number.

CCD, cross-reactive carbohydrate determinant; LP, *Lolium perenne*; OE, *Olea europaea*; HBV, *Apis mellifera* (honey bee) venom; YJV, *Vespa* spp. (yellow jacket) venom; DP, *Dermatophagoides pteronyssinus*; ND, not done; sIgE, specific IgE; SPT, skin prick test.

titres were exceedingly high, with values frequently surpassing 3.5 kU/L (class 3) or even 50 kU/L (class 5). It seems likely that the presence of sIgE to Hymenoptera venoms in heavy drinkers is related to CCD interference because (a) in most cases, there was double positivity (i.e. sIgE to both *A. mellifera* and *Vespa* spp.), which is a characteristic feature of CCD interference [29, 31, 34], (b) titres of CCD-sIgE were particularly high in double-positive cases, (c) there was a strong correlation between sIgE to Hymenoptera venoms and CCD-sIgE positivity, and (d) sIgE reactivity to Hymenoptera venoms was inhibited by pre-incubation with CCD in some of the selected cases. However, both correlation and inhibition were weaker and less consistent than that observed for pollens. Nearly all individuals with sIgE to CCD had an equal or higher level of sIgE to Hymenoptera venoms, but there were individuals with very low levels of CCD-sIgE and a high level of sIgE to these venoms. This difference with pollens could be expected because glycans from Hymenoptera glycoproteins contain core α 1,3-fucose but not xylose [26–28]. Taken together, these results indicate that heavy drinkers may be sensitized not only to MUXF³ but also to additional CCDs or non-CCD epitopes.

The mechanisms for the increased prevalence of CCD-sIgE in relation to heavy drinking are not known. The association was still present after adjusting for known confounders. It seems unlikely that non-specific IgE

binding due to increased total serum IgE is responsible for CCD reactivity because there were many subjects with normal IgE levels and positive CCD-sIgE (data not shown) [32]. Furthermore, increased sIgE in heavy drinkers appeared to be dependent on the CCD content of allergens. Hypothetically, CCD-sIgE in heavy drinkers could derive from an increased CCD exposure due to alcohol-induced increased permeability of the respiratory [35] or the gut [36] mucosa. Also, alcohol intake may decrease protein digestion in the gut, thus favouring sensitization [9, 37]. Smoking is associated with alcohol consumption and can enhance respiratory permeability for allergens [38]. However, the effect of alcohol on CCD-sIgE was independent of smoking, which had no effect on CCD-sIgE positivity by itself. Interestingly, heavy drinkers also show significant aberrations in self-protein glycosylation that include desialylation [39] and fucosylation [40]. These intriguing hypotheses about the source of CCD exposure in heavy drinkers warrant further study.

Current thought is that the presence of sIgE to CCDs is of minor clinical importance [15, 16]. However, there are *in vitro* indications that CCD-sIgE may have biologic activity, as reviewed recently [15]. Moreover, alcohol consumption is associated with a trend towards a higher rate of positive SPT to respiratory [6, 7] and food [41] allergens. Thus, further studies of the biological relevance and prognostic value of CCD-sIgE in heavy drinkers are also warranted.

Methodological issues should be considered. The CCD used (MUXF³, derived from bromelain) is widely used for checking glycan-induced cross-reactivity because of its widespread distribution, and because it contains the oligosaccharide substitutions that are crucial for the antigenic properties of glycans [15–17, 21, 22]. Here, we used the isolated glycan determinant instead of the complete bromelain molecule in order to gain specificity. However, this can induce a lower sensitivity in CAP assays, according to the manufacturer's instructions. If a CAP test to bromelain or MUXF³ is positive, it means that the patient's serum contains CCD-sIgE [16]. Nevertheless, the IgE-reactive *N*-glycosidic epitope is not a single uniform entity because there are many forms of glycan in nature and there is not a single test to detect CCD-sIgE [15, 16]. Preliminary results in a different population sample indicate that heavy drinkers have a similarly increased prevalence of sIgE to ascorbate oxidase, which contains an MMXF-type CCD (unpublished observations).

In summary, our study presents novel findings about the prevalence of CCD-sIgE in the general adult population, and its association with heavy drinking. The results are of importance because the presence of CCD-sIgE is linked to positivity of sIgE to pollens and Hymenoptera venoms, probably due to cross-reactivity. According to our experience, a questionnaire on alcohol consumption should be a routine part of the standard allergological evaluation. Furthermore, sIgE results should be interpreted with special caution in heavy drinkers.

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