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Immunoglobulin-E reactivity to wine glycoproteins in heavy drinkers

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Abstract

N-glycans from plant and invertebrate allergens can induce extensive immunoglobulin-E (IgE) cross-reactivity in vitro. IgE antibodies against these N-glycans, also termed cross-reactive carbohydrate determinants or CCDs, are prevalent in alcohol drinkers. This study investigated the prevalence and biological significance of IgE antibodies to N-glycans from wine glycoproteins in heavy drinkers. A structured questionnaire, skin prick tests, serum IgE levels, IgE-immunoblotting to wine extracts, and basophil activation tests were used to characterize 20 heavy drinkers and 10 control subjects. Eleven heavy drinkers (55%) showed IgE binding to proteins in wine extracts. The proteins were identified by mass spectrometry as grape-derived vacuolar invertase and thaumatin-like protein. Immunoblot reactivity was closely associated with the presence of IgE to CCDs and was inhibited by preincubation with a glycoconjugate containing bromelain-type N-glycans. The same conjugate, CCD-bearing allergens, and wine extracts activated basophils in patients with high-titer CCD-specific IgE but not in healthy controls. There was no relationship between immunoblot reactivity and consumption of any specific type of wine. No patient reported symptoms of hypersensitivity to Hymenoptera venom, food, or wine. In conclusion, heavy drinkers frequently show IgE reactivity to the N-glycans of wine glycoproteins. Glycans and wine glycoprotein extracts can induce basophil activation in sensitized alcoholics. The clinical significance of these findings remains to be elucidated.

Keywords: Immunoglobulin-E; Wine; Cross-reactive carbohydrate determinants; Glycans; Allergy; Basophil activation

Introduction

Immunoglobulin-E (IgE) is the molecule responsible for allergic hypersensitivity. Allergen-induced cross-linking of IgE on the surface of mast cells and basophils induces the release of mediators of allergic inflammation (Hamilton, 2010). Determination of IgE specific to allergens is routinely used for the diagnosis of allergic disease (Hamilton, 2010), and allergen-mediated basophil activation tests are performed in selected cases (De Week et al., 2008). The ability of an allergen to trigger the activation of basophils indicates that allergen-specific IgE has biological activity in that patient (De Week et al., 2008; Hamilton, 2010). Of note, in vitro IgE reactivity or basophil activation are not always accompanied by in vivo activity or clinical allergy. Allergenic cross-reactivity should be taken into account when interpreting the results of allergy tests. Primary sensitization mediated by IgE against a particular allergen can induce reactivity to structurally related molecules (Aalberse et al., 2001). Along this line, many plant and invertebrate glycoproteins contain similarly fucosylated and/or xylosylated N-glycans, also known as cross-reactive carbohydrate determinants (CCDs) (Altmann, 2007; Malandain, 2005; Mari, 2002; van Ree, 2002). Because CCDs are ubiquitous in nature, CCD sensitization can induce widespread IgE reactivity, therefore limiting the specificity of in vitro tests for allergy (Altmann, 2007; Malandain, 2005; Mari, 2002; van Ree, 2002).

Previous studies revealed an increased rate of IgE sensitization to CCDs in alcohol drinkers (Coutinho et al., 2008; Gonzalez-Quintela et al., 2008; Vidal et al., 2009), although the mechanisms and biological significance of this are unknown. Alcohol (ethanol) is a powerful immunomodulator that may shift immunity toward Th2 responses (Heinz and

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Waltenbaugh, 2007; Linneberg et al., 2008; Szabo, 1999). In fact, alcohol consumption is associated with increased serum total IgE levels in both observational studies in humans (Gonzalez-Quintela et al., 1995, 2003; Hallgren and Lundin, 1983; Linneberg et al., 2003; Vidal et al., 1994) and experimental studies in rodents (Andrade et al., 2006; Heinz and Waltenbaugh, 2007; Linneberg et al., 2008). In theory, additional factors for CCD exposure and subsequent sensitization in alcohol drinkers could include the glycoprotein content of alcoholic beverages. Grapes and wines contain proteins that in rare cases can induce allergic sensitization (Pastorello et al., 2003; Vassilopoulou et al., 2007). Wine proteins are derived mainly from grapes (*Vitis vinifera*) (Cilindre et al., 2008; Ferreira et al., 2001; Yokotsuka and Singleton, 1997), although some may come from yeasts that induce wine fermentation (Dambrouck et al., 2003). Some wine proteins are glycosylated (Ferreira et al., 2001); interestingly, the glycan content of wine glycoproteins is higher in young wines than in older wines (Yokotsuka and Singleton, 1997). In addition, it has been suggested that Hymenoptera allergens may be present in wines (particularly in young wines) because honeybees or wasps may be crushed along with grapes during the initial steps of winemaking (Armentia et al., 2007). The presence of Hymenoptera allergens in wine could be relevant because exposure to Hymenoptera stings may be a factor for CCD sensitization (Aalberse et al., 1981; Kochuyt et al., 2005). Rural areas in northwestern Spain have a long tradition of viticulture, and consumption of young homemade wines is very common (Mateos et al., 2002). The present study was aimed to investigate the frequency, associated factors, and the biological significance of IgE antibodies to N-glycans (CCDs) of wine glycoproteins in heavy drinkers.

Methods

Participants

This study included 20 heavy drinkers consecutively admitted to the Internal Medicine Department of a University Hospital in Northwest Spain (2007–2008) because of alcohol withdrawal syndrome ($n = 9$), alcoholic hepatitis ($n = 7$), complications of advanced liver disease ($n = 3$), and cerebrovascular disease ($n = 1$). All patients were predominantly wine consumers, with 10 consuming primarily homemade young wines. Thirteen patients also consumed beer, and nine also included spirits. Ten healthy volunteers with an alcohol intake of less than 1 unit/d were included as controls. Patients and controls were comparable in epidemiological factors (Table 1). The study was approved by the Regional Ethics Committee. Written informed consent was obtained from all participants.

Questionnaire

A physician-administered questionnaire included tobacco consumption, residence, occupation, respiratory symptoms, symptoms of food allergies, oral allergy syndrome, lifetime history of Hymenoptera stings, and Hymenoptera venom allergy. The average daily alcohol consumption was expressed in grams. For that purpose, we registered the number of drinks (glasses of wine, bottles of beer, and drinks of spirits) consumed per day. Validation of the standard drinking unit (SDU) system in Spain indicates that drinks of wine and beer are approximately equivalent to one SDU or 10 g of alcohol, and drinks of spirits are approximately equivalent to two SDUs or 20 g of alcohol (Gual et al., 1999).

Liver function tests

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglutamyltransferase (GGT), and bilirubin were assayed in an Advia analyzer (Siemens Medical Solutions Diagnostics, Munich, Germany). The upper normal limit for serum AST, ALT, and GGT is 25, 29, and 32 IU/L, respectively. The prothrombin time was expressed as the international normalized ratio.

Glycoconjugates

Neoglycoproteins (MUXF-BSA and MM-BSA) consisting of N-glycopeptides conjugated to bovine serum albumin (BSA) were provided by the Institute of Chemistry, Universitaet fuer Bodenkultur, Vienna, Austria. MUXF (the N-glycan from bromelain) has, like most CCDs, a backbone with two N-acetylglucosamines and a mannose. "M" indicates the presence of a terminal α-mannose, "U" indicates an unsubstituted 3-hydroxyl on the β-mannose, "X" indicates a xylose, and "F" indicates the presence of a core α1,3-fucose. MM-BSA contains similar N-glycans with an additional mannose but lacking the xylose and fucose that are crucial for the antigenic properties of CCDs, as reviewed by Altmann (2007).

Serum IgE and inhibition studies

Total IgE was measured by chemiluminiscent immunoassay (Immulite-2000, Siemens Medical Solutions, Gwynedd, UK). Allergen-specific IgE was assayed using the UniCAP-250 system (Phadia, Uppsala, Sweden). Determinations included IgE to grapes (*V. vinifera*, f259), honeybee venom (*Apis mellifera*, i1), common wasp (yellow jacket) venom (*Vespula* spp., i3), grass pollen (*Lolium perenne*, g5), olive tree pollen (*Olea europaea*, t9), peanuts (*Arachis hypogaea*, f13), latex (*Hevea brasiliensis*, k82), and two CCD markers (MUXF [Ro214] and bromelain itself [*Ananas comosus*, k202]). The reportable range of the CAP method is 0.01–100 kU/L. As per the manufacturer's instructions, IgE levels greater than or equal to 0.1 kU/L were considered to be "positive" in some analyses.

CAP inhibition by CCD was performed in individuals with specific IgE levels greater than or equal to 0.35 kU/L (the classic threshold for positivity) using MUXF-BSA as inhibitor, as previously described (Gonzalez-Quintela et al.,