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Cross-reactive carbohydrate determinants (CCDs)

Introduction:

Most of the proteins in all species are glycoproteins and often found on the outer surface of cells. Glycoproteins consist of a protein or peptide part and glycan-chains also termed carbo-hydrates. These glycan-chains are made from different sugars (like mannose, galactose, N-acetylglucosamine, fucose and xylose) and linked via an amino-group (N-glycan) or via a hydroxyl-group (O-glycan) to the protein part [1]. N-glycans from plants and insects deviate in several ways in structure from human N-glycans. Two main motifs are the core α1,3-fucose residue and a xylose-residue, while plants contain both motifs, insect venoms only contain the fucose residue [2-6]. These glycan-chains have the potency to induce the production of im-munoglobulin E (IgE), which is highly cross-reactive to glycoproteins of plant and insect ori-gin [7-8]. Glycan-chains are therefore called cross-reactive carbohydrate determinants (CCDs) [7].

Occurrence:

Extracts made from natural plant and insect allergens contain cross-reactive carbohydrate de-terminants. This is true for extracts made from: tree, weed and grass pollen, vegetables, fruits, seeds, latex and insect venoms [9-12].

Role in allergy diagnostics:

Approximately 20% of allergic persons produce anti-glycan-specific IgE and IgG [13-14]. Although CCDs do not emerge to cause clinical symptoms, their presence has to be consid-ered in allergy diagnostics. While there is no positive result in skin prick tests, in *in vitro* test systems the occurrence of CCDs can lead to *false-positive* results or to an exaggeration of a positive result [15-17]. In the first case anti-CCD specific IgEs are present in the patient se-rum but no specific IgE for the protein part of the allergen. In the second case specific IgE for the protein part of the allergen and CCD-specific IgE are present in the patient serum.

There are two models to explain the discrepancy between *in vitro* test results and *in vivo* test results. In an *in vitro* test system a monovalent binding of IgE to the allergen is sufficient for a

positive result. But for an *in vivo* effect the cross-linking of IgE to an at least bivalent allergen is necessary to trigger degranulation of mast-cells [1]. However, most of the known glycan-allergens just carry one glycan-chain and therefore are considered to be monovalent [1].
A second assumption is based on the finding, that many allergic patients with glycan-specific IgE also contain CCD-specific IgG4 antibodies or blocking antibodies [1, 18]. The prevention of IgE binding to an allergen by blocking antibodies is an important feature of a specific immunotherapy (SIT) [19-20]. Because people have contact to plant material almost every day, many people undergo a natural immune therapy (glycan-SIT) [1].

Neutralisation of CCDs:

To minimize the risk of misdiagnosis of food, latex or insect venom allergy especially in pollen allergic patients it is necessary to eliminate the reactivity of anti-CCD IgE [1]. A good strategy would include the detection of serum containing anti-CCD IgE through the usage of a plant glycoprotein as an additional allergen. When a serum was detected positive for anti-CCD IgE the next step is to eliminate the reactivity of anti-CCD IgE. This can be achieved by adding an inhibitor to the serum. It is not suffice to detect anti-CCD IgE containing serum. For a reliable allergy diagnostic it has to be distinguished between a sensitisation to CCD´s and a true or peptide based sensitisation [1]. The presence of anti-CCD antibodies will lead to many positive tested allergens because of the glycan-IgE interaction, but it does not rule out the possibility of peptide-antibodies, which are capable of triggering clinical symptoms.

Procedure:

With the AllergyScreen®/AlleisaScreen® test-systems it is possible to block anti-CCD IgE with the CCD solution. This solution consists of a mixture of bromelain, horseradish peroxidase and ascorbate oxidase. These three plant proteins contain different glycan-chains with a high affinity for anti-CCD IgE [3, 9, 21-23]. The serum is supplemented with the CCD solution, thoroughly mixed and incubated at room temperature for 1hour. Therefore one third of the CCD-solution is given to two thirds of the serum. During the incubation step anti-CCD antibodies bind to the glycan-parts of the proteins. After the pre-incubation of the serum and the CCD solution the serum-CCD mixture can be used in the AllergyScreen® and in the AlleisaScreen® assays to test for allergen-specific IgE antibodies.

	CCDs: cross-reactive carbo-hydrate determinants	N. Hofmann
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Example:

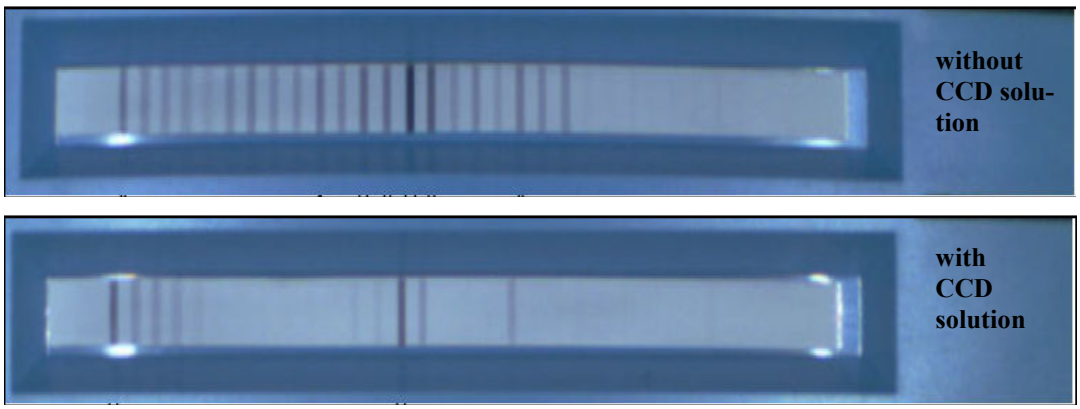

Figure 1: Shown is an example of a patient serum tested without the CCD solution (top) and the same serum after preincubation with the CCD solution (bottom). There is a significant decrease in positive tested allergens after the preincubation with the CCD solution. Hence a couple of strong lines are still visible. These lines represent allergens, where a true sensitisation of the patient exists.

Table 1: Given are the measured results in classes of the two test strips shown above.

	CCD solution			CCD solution	
Allergen	without	with	Allergen	without	with
D1	3,3	3,3	W20	3,5	0,0
D2	3,2	3,2	W206	3,0	0,0
D70	3,0	3,1	I1	3,8	3,1
I6	2,7	2,3	I3	3,4	0,0
T2	3,3	0,0	K82	3,4	0,5
T3	3,6	0,0	M2	2,0	0,8
T4	3,3	0,0	M6	0,0	0,0
T7	3,2	0,0	M1	0,8	0,0
Gx	3,6	0,4	M3	0,0	0,0
G12	3,3	0,0	E1	0,0	0,0
W1	4,3	2,2	E3	0,0	0,0
W2	4,1	2,5	E5	2,0	1,6
W6	6,0	5,2	E6	0,0	0,0
W7	4,8	3,3	E82	0,0	0,0
W9	3,1	0,0	E84	0,0	0,0

Interpretation:

After the preincubation of the serum with the CCD solution it becomes apparent that some of the positive tested allergens are *false-positive*, for example I3 wasp venom and K82 Latex as well as several grass and tree pollen. These allergens are tested positive because of the glycan-antibody interaction but there is no peptide-antibody interaction. However, the peptide-antibody interaction is responsible for clinical symptoms of an allergy.
Still some allergens are tested positive in both cases, like I1 bee venom and E5 dog epithelium, demonstrating that a true or peptide based sensitisation is not affected from the CCD solution. In some cases (W1, W2, W7) there is a decrease in the measured classes when the CCD solution is used. This happens when anti-CCD antibodies and allergen-specific antibodies are present in the serum. The CCD solution blocks the binding of the anti-CCD antibodies but the peptide-specific antibodies once more bind to the allergen, resulting in a positive but decreased result.

Conclusion:

Cross-reactive carbohydrate determinants are widespread in allergen extracts made from natural food, tree and grass pollen, weeds, insect venoms and latex. Approximately 20% of allergic patients have anti-CCD IgE antibodies. These antibodies bind to any allergen with a glycan-part. This binding can lead to *false-positive* results and it is difficult to distinguish between a true sensitisation and a glycan related sensitisation. Through the use of glycoproteins like bromelain, horseradish peroxidase and ascorbate oxidase as additional allergens in the AllergyScreen® or AlleisaScreen® system it is possible to detect anti-CCD IgE containing sera. These sera should be treated with the CCD solution to eliminate the reactivity of the anti-CCD IgE and afterwards tested again with the AllergyScreen® or AlleisaScreen® system. This procedure improves the allergy diagnostic and makes it possible to reduce the misdiagnosis of food, latex or insect venom allergies.

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