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REVIEW

N- and O-linked oligosaccharides of allergenic glycoproteins

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Cross-linking of cell-bound IgE on mast cells or basophils by polyvalent antigens causes the release of histamine and other mediators of the allergic response which then lead to the development of allergic symptoms. In this event not only peptide epitopes, but also carbohydrates can act as cross-linking elements. Since peptide epitopes of allergens are subject of most published studies, this review is focused on glycosidic epitopes. The current knowledge of the structures and possible epitopes of oligosaccharides linked to allergenic glycoproteins is briefly reviewed, showing that complex plant N-glycans containing α1,3 fucose and β1,2 xylose are most frequently involved in the structures of IgE epitopes. In own studies a prevalence of up to 29% anti-glycan IgE was determined among pollen-allergic patients. The clinical relevance of these carbohydrate specific IgE antibodies is still a matter of controversial discussions.

Keywords: **CCD, IgE reactivity, epitope, prevalence, clinical relevance**

Introduction

Mast cells and basophils can bind immunoglobulin E (IgE) antibodies to receptors on their surface. Aggregation of these receptors by contact of cell-bound IgE with multivalent antigen leads to the rapid extracellular release of histamine, heparin, proteases and other mediators that are stored in the cell's cytoplasmic granules, and the synthesis and secretion of leukotrienes and prostaglandines. Finally, these products result in appearance of allergic symptoms [1]. The most important prerequisite for the initiation of the mediator release is the polyvalence of the antigen (Figure 1, [refs. 2,3]), because monovalent antigens, that means antigens with a single epitope, are unable of cross-linking of cell-bound IgE (Figure 1A). The nature of the epitopes is not necessarily peptidic (Figure 1B, right side), but can also be of glycosidic origin (Figure 1C).

In 1972 Lee and Scocca [4] investigated the glycan structures of the three allergenic glycoproteins bromelain, ovalbumin and α-amylase. In all three cases they found the glycan Manβ1,4GlcNAcβ1,4GlcNAc linked to the asparagine residues of the polypeptide. This structure was later been shown to be the common core structure of many plant and animal glycoproteins (see Figure 2). It took, however, another seven years before the first N-linked glycan structure of a plant glycoprotein, the cysteine protease bromelain was correctly resolved [5]. Due to the complexity of the matter and the laboratory equipment and know-how required, many papers of that time dealt and deal even nowadays only with the determination of the carbohydrate contents [6,7] or with the carbohydrate composition of the allergenic glycoproteins [8–10], but in many cases the whole glycan structures could not be resolved. As a consequence, more and more structures of N-linked glycans of allergenic glycoproteins became available only very recently, starting in the late eighties (see Table 1, [refs. 5, 11–41]). Lately, many papers were published with new results concerning the glycan structures of whole allergenic plant extracts [15,34,35,40] or single allergens [36,37,39,41]. Therefore, this paper will briefly review the recent knowledge of the N- and O-linked oligosaccharides of allergenic glycoproteins and their impact on IgE binding.

Structures of N- and O-linked oligosaccharides

Although the results of several studies on Asn-linked glycans suggest that there are possibly thousands of structurally different oligosaccharides, these were shown to possess common structural features. The common characteristics are

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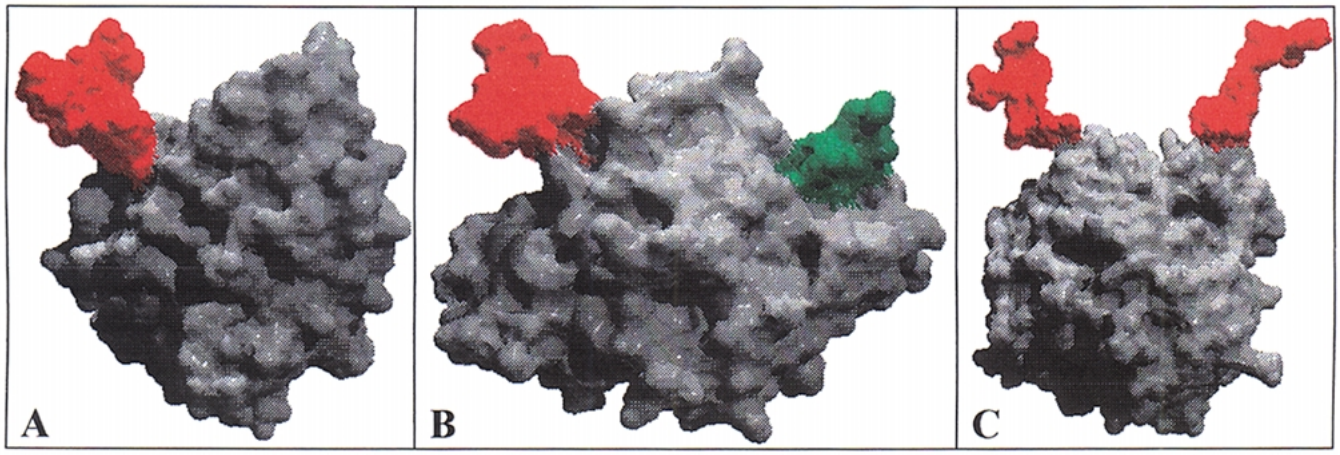

Figure 1. Display of mono- and polyvalent antigens (possible carbohydrate epitopes depicted in red, protein epitope depicted in green). A: monovalent antigen: only one carbohydrate epitope (branch structure), unable for cross-linking of IgE. B: divalent antigen: one carbohydrate (red) and one protein epitope (green) C: divalent antigen: two carbohydrate epitopes. The pictures were prepared with Swiss-PdbViewer v3.6b3 using the structures of A+B: Erythrina corallodendron lectin (PDB-No. 1AX0, [2]) and C: Neuraminidase N2 (PDB-No. 2BAT, [3]) and were rendered with Pov-ray v3.1g.

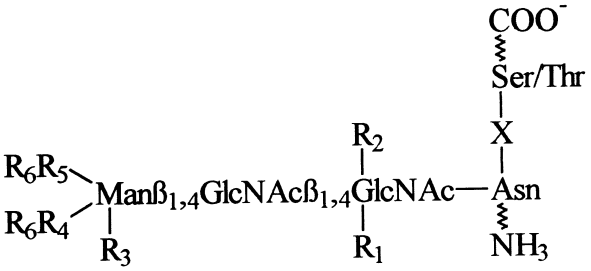

Figure 2. Simplified scheme of the structures of N-linked oligosaccharides. High mannose-type oligosaccharides: R1–R3 = –OH, R4 and/or R5 = Man, R6 = Man and/or Glc. Hybrid-type oligosaccharides: R1 and/or R2,R3,R6 ≠ –OH or Man, R4 and R5+R6 = Man. Complex-type oligosaccharides: R1 and/or R2,R3,R6 ≠ –OH or Man, R4 and/or R5 = –OH or Man.

depicted in Figure 2: firstly, the existence of the already mentioned common trisaccharide core and secondly, the linkage of this core structure to the asparagine residue in the consensus sequence -Asn-X-Ser/Thr- of the peptide chain.

Furthermore, according to their main structural features N-glycans can be divided into three major types: high mannose-type, hybrid-type, and complex-type (Figure 2; for review see [42,43]).

Examples of all three classes are given in Table 1 showing the nonreducing termini of the structures evaluated on allergens or antigens and therefore also the possible carbohydrate epitopes for anti-carbohydrate antibodies. For simplification, the truncated 'paucimannosidic' structures (shorter than 5 mannose residues) were included in their respective classes. The table also illustrates the development of sophisticated methods in analytical glycobiology, since more and different oligosaccharides per allergen/extract could be investigated in the recent years [34,40]. Simultaneously, some earlier published structures were corrected. So, the revised analysis of the glycan of bromelain [11] showed that the second glycan structure found by Ishihara et al. [5] with a linear array of three mannose residues does not exist.

Wilson et al. [40] could detect hybrid-type oligosaccharides in many food extracts, structures formerly thought to be rather uncommon on allergenic glycoproteins. These oligosaccharides could partly account for large amounts of the N-linked oligosaccharides of different foods (up to 75% in Papaya). Besides, also Lewis A (Le^a) determinants (Galβ1-3[Fucα1-4]GlcNAc) were found in many plant extracts (up to 51% in apple) and constitute another possible source of cross-reactivity [35,40,41]. This structure was also detected in the *N*-linked glycans of sycamore cell laccase [15], thereby correcting the wrong finding of Takahashi et al. [14], who described α1,6-linked fucose as terminal sugar residue of the laccase glycan. As well, the assignment of α1,6-linked fucose residues to the *N*-glycans of *Cry j* I [29,30] might be wrong. Instead, it seems probably more accurate to conclude that *Cry j* I carries the Le^a epitope with α1,4-linked fucose [41].

Furthermore, Altmann [17] could show that a large proportion of the carbohydrates of ascorbic oxidase (57%) contains α1-3-linked fucose. This contrasts to the longtime opinion and use of ascorbic oxidase as only xylose-containing glycoprotein due to the study previously reported by D'Andrea et al. [16] on ascorbate oxidase from a non-commercial source.

O-linked glycans of plant allergens consist in most cases of single sugar residues forming long carbohydrate chains, the "backbone" of the structure, to which side chains formed by other or of the same monosaccharides are attached (e.g. arabinogalactans, which have a backbone of β1,3-linked galactose residues). These structures often have a high molecular weight, and are linked to serine or threonine or

hydroxyproline residues of the polypeptide (for review see [44]). Hydroxyproline residues can also be linked to arabinose residues via O-glycosidic bonds [45] which could thus form further potential IgE binding moieties (Table 2, [refs. 46–62]).

Unlike the O-linked glycans of plants are those of sea squirt allergens, which possess a trisaccharide core structure that consists of GalNAcβ1-4GlcNAcβ1-3GalNAc. This core can be modified by fucose and/or GlcNAc and/or further GalNAc residues (see Figure 3).

Determination of IgE reactivity to oligosaccharides

Many papers dealing with the structural analysis of the carbohydrate moieties of allergenic glycoproteins only showed the general binding of the isolated glycoprotein to the IgE of one or more allergic patients, but not the binding of IgE to the pure glycan [7,8]. Other studies demonstrated the presence of carbohydrates on various allergens either by lectin binding or by antibodies specific for certain sugar residues [63–65]. These studies supplied clues for additional IgE epitopes on the glycoproteins being formed by the glycans, but no direct evidence for their involvement in allergenicity.

One of the first groups conducting experiments to show the impact of the carbohydrate moiety on the antigenicity of the extract were Ishii et al. [66]. They investigated the carbohydrate composition of the extract of the house dust mite *Dermatophagoides farinae*, used the extract in skin tests in 10 asthmatic patients and treated it by heating, by trypsin and pronase digestion and also by periodate oxidation. The treated extracts were not tested with human sera, only with sensitized guinea pigs, where the strongest reduction of the Prausnitz-Kuestner type skin reactions was obtained with periodate-treated extracts [66].

Aalberse et al. [67] were the first who showed that periodate oxidation of grass pollen, peanut and buckwheat extracts strongly reduced the IgE binding of sera from three atopic patients to these extracts. These results were also confirmed by RAST inhibition experiments with periodate treated potato extract (Table 3). Due to the high cross-reactivity of such IgE antibodies to many plant extracts these authors also introduced the term "CCD" for "cross-reactive carbohydrate determinants". Later, the ubiquitous occurrence of such CCD in plants was repeatedly proven [34,63,65,68–71].

Thereafter, more and more studies showed that glycan moieties of allergenic glycoproteins take part in the induction of an IgE response in allergic patients. Table 3 summarizes the results of selected publications dealing with the IgE reactivity of the glycans of the allergens under investigation [refs. 10,22,24,32,37–39,47,48,50,53,55,57–59,62,67,69–117]. In most cases evidence for IgE binding resulted from periodate oxidation of the (blotted) extracts or allergens, or by trifluoromethanesulphonic acid (TFMS), or protease-treatment, or by IgE inhibition assays using defined glycan structures as inhibitors. Figure 4 shows examples of blot inhibitions with such purified glycans using a CCD-positive patient tested on three different plant extracts. The IgE of this patient detects many bands in the high molecular weight range indicating the presence of glycoproteins in all extracts (lane 1). In the presence of the purified bromelain glycan (lane 2) or of the extracts containing similar glycan structures on their glycoproteins (lanes 5–7), the IgE binding to high molecular weight bands was totally abolished. In contrast, no inhibition at all was achieved with either the fibrin glycan, which contains no fucose and xylose residues (lane 3), or with the non-glycosylated recombinant major birch pollen allergen Bet v 1 (lane 4).

In other experimental strategies, the IgE binding to carbohydrate structures was concluded from ELISA measurements using Proteinase K-digested extracts or defined glycopeptides as solid phase antigens, or by comparison of the IgE reactivity of glycoproteins with unglycosylated variants thereof or corresponding recombinant proteins expressed in *E. coli.* By contrast, treatments with glycosidases were less successful in certain cases, as they led to incomplete deglycosylations of the allergens and thus produced non-utilizable results (see Table 3). Also it is known that periodate oxidation can alter the amino acids of the peptide chain [118] and thus leads to a reduced IgE binding because of the loss of peptidic epitopes. Therefore, this treatment has to be carried out under mild and carefully controlled conditions, which in most studies was well documented. An alternative chemical deglycosylation method is to use trifluoromethanesulphonic acid; however, in three cases, this treatment not only removed the glycans from the protein, but also affected the secondary structures of Par j 1 [81], of Fel d 1 [84] and of Ole e 1 [88] significantly, as demonstrated by changes in the circular dichroism (CD) spectra of the treated allergens. This is in contrast to earlier studies which claimed nearly no, or only minimal effects, on the structural integrity of the polypeptide chains by TFMS treatment [119,120]. Nevertheless, even a partial loss of protein conformation could strongly influence the IgE binding and result in misleading data. In two of these three cases [84,88] also other deglycosylation methods were used to confirm the CCD binding. However, in most of the other studies summarised in Table 3 the conservation of the overall secondary structure of the polypeptide chains after TFMS treatment was not investigated.

Epitope characterization

Table 1 reveals that the non-reducing termini of N-linked glycans potentially involved in antibody binding consist mainly of mannose, fucose, xylose and N-acteyl-glucosamine in different linkages. Several studies were undertaken to test the influence of these main structural elements on the IgE binding. In an own study, a carbohydrate ELISA with defined glycan structures covalently linked to the surface of microtitre plates [100,102] was used to analyze sera of around 300 patients allergic to tree and grass pollen for IgE binding to

Table 1. Structures of N-linked glycans presumably involved in antibody binding (nonreducing termini)

		1. High-Mannose-Type			*2. Hybrid-Type*				*3. Complex-Type*[1]							
Allergen	*Source*	*No.**	*R4-R6 + Core Man*	*R6*[2] *other*	*No.**	*R4-R6 +Core Man*	*R3 other*	*R6 other*	*No.**	*R1 F1-3*	*R3 X1-2*	*R3 other*	*R4/R5 Man*	*R6 GlcNAc*	*R6 other*	*Reference*[3]
Bromelain	Pineapple	-			-				2	++	++		++		Man	[5]
Bromelain	Pineapple	-			-				1	+	+		+			[11]
Ovotransferrin	Egg White	-			-				2			GlcNAc		++		[12]
OVA/Ovomucoid	Egg White	2	M5+M6		4	M4+M5	GlcNAC	GlcNAC + Gal	2			GlcNAc		++	Gal	[12]
OVA	Egg White	2	M5+M6		1	M5	GlcNAc	GlcNAc	-							[13]
Laccase	Sycamore	-			-				6	++	++		+	+	F1-6 + Gal[5]	[14]
Laccase	Sycamore	-			-				4	++	++		+	+	F1-4 + Gal	[15]
Ascorbate oxidase	Zucchini	-			-				1		+		+			[16]
Ascorbate oxidase	Zucchini	-			-				4	+	++		++	+		[17]
Peroxidase	Horseradish	-			-				1	+	+		+			[18]
Peroxidase	Horseradish	-			-				1	+	+		+			[19]
α-Amylase	Rice	6	M5-M9	Glc	-				4		+		+	+		[20]
β-Fructosidase	Carrot	1	M7 or M8		-				2	++	++		+	+	Ara	[21]
Art v 2	Mugwort	5	M5-M9		-				-							[22]
Peroxidase BP1	Barley seed	-			-				1	+	+		+			[23]
PLA_2	Honeybee venom	1	M4		-				2	+		R2 = F1-6	+		GalNAc + F1-3	[24]
PLA_3	Honeybee venom	4	M2-M4		-				10	+		R2 = F1-6	++		GalNAc + F1-3	[25]
Hyaluronidase	Honeybee venom	7	M1-M6, M9		-				11	++		R2 = F1-6	++		GalNAc + F1-3	[26]
Free N-Glycans	Tomato	7	M5-M8		-				3	+	+	Ara	+	+		[27]
N-linked Glycans	Tomato	6	M3, M6-M9		-				10	++	++		++	+		[28]
Cry j 1	Crypt. japonica	-			-				4	++	(+)			++	F1-6 + Gal[4]	[29]
Cry j 1	Crypt. japonica	-			-				4	++	++			+	F1-6 + Gal[4]	[30]
BG 60	Bermuda grass	-			-				5	++	(+)		+	+		[31]
BG 60	Bermuda grass	-			-				1	+			+			[32]
Equ c 1	Horse	-			-				2						Sialic acid	[33]
N-linked Glycans	Kentucky blue grass	-			-				7	++	++		++	+		[34]
N-linked Glycans	Rye	-			-				7	++	++		++	+		[34]
N-linked Glycans	Rye grass	-			-				9	++	++		++	+		[34]
N-linked Glycans	Giant Ragweed	-			-				8	++	+		++	+		[34]
N-linked Glycans	Short Ragweed	-			1	M4		Fuc1-3	6	++	+		++	+		[34]
N-linked Glycans	Birch	3	M7-M9		-				6	++	++		+	++		[34]
N-linked Glycans	Horse Chestnut	5	M5-M8		-				6	+	+		+	+		[34]
N-linked Glycans	Hornbeam	5	M5-M8		-				4	+	+		+	+		[34]
N-linked Glycans	Pine	2	M7+M8		-				4	++	++		+	++		[34]
N-linked Glycans	Olive	5	M6-M9		-				7	+	++		+	+		[34]
N-linked Glycans	Tobacco	-			-				5	++	++		+	+	F1-4+Gal	[35]
Ole e 1	Olive tree	4	M5-M8		-				6	(+)	++		+	++		[36]
Ole e 1	Olive tree	1	M7		-				2	(+)	++		++	++		[37]

Ara h 1	Peanut	3	M4-M6		-				2		++	++			[36]
Ara h 1	Peanut	2	M5+M6		-				2		++	++			[37]
Lol p 11	Rye grass	-			-				4	++	+	++	++		[37]
48 kDa Allergen	Hazelnut	1	M5		1	M4	Xyl1-2		2	(+)	++	++			[38]
Polyamine oxidase	Maize	-			-				1	+	+	+			[39]
N-linked Glycans	Almond	6	M4-M9		1	M4	Xyl1-2	GlcNAc	10	++	++	++	(+)	F1-4+Gal	[40]
N-linked Glycans	Apple	7	M4-M9	Glc	1	M4	Xyl1-2	Fuc1-3	13	++	++	+	+	F1-4+Gal	[40]
N-linked Glycans	Asparagus	5	M6-M9	Glc	-				13	++	++	+	+	F1-4+Gal	[40]
N-linked Glycans	Avocado	6	M5-M9	Glc	2	M4	Xyl1-2	GlcNAc + Fuc1-3	13	+	+	+	+	F1-4+Gal	[40]
N-linked Glycans	Banana	5	M6-M9	Glc	-				14	++	++	+	+	F1-4+Gal	[40]
N-linked Glycans	Buckwheat	6	M5-M9	Glc	2	M4	Xyl1-2	GlcNAc	11	+	+	+	+	F1-4+Gal	[40]
N-linked Glycans	Carrot	5	M6-M9	Glc	1	M4	Xyl1-2		10	+	+	+	+	F1-4+Gal	[40]
N-linked Glycans	Cauliflower	6	M5-M9	Glc	1	M5	Xyl1-2	GlcNAc	7	++	++	+	+	Gal	[40]
N-linked Glycans	Celery	5	M5-M9		1	M4	Xyl1-2	GlcNAc	14	++	++	+	+	F1-4+Gal	[40]
N-linked Glycans	Coconut	4	M5-M8		2	M4	Xyl1-2	GlcNAc	5	(+)	++	++	(+)	F1-4+Gal	[40]
N-linked Glycans	Hazelnut	5	M5-M9		-				15	++	++	+	+	F1-4+Gal	[40]
N-linked Glycans	Mung bean	7	M4-M9	Glc	-				4	(+)	(+)	(+)	(+)	F1-4+Gal	[40]
N-linked Glycans	Mushroom	6	M5-M9	Glc	-				-						[40]
N-linked Glycans	Kiwi	5	M5-M9		1	M4	Xyl1-2		15	++	++	+	+	F1-4+Gal	[40]
N-linked Glycans	Onion	5	M6-M9	Glc	-				14	++	++	+	+	F1-4+Gal	[40]
N-linked Glycans	Orange	5	M6-M9	Glc	-				12	++	++	+	+	F1-4+Gal	[40]
N-linked Glycans	Papaya	4	M5-M7,M9		9	M4+M5	Xyl1-2	GlcNAc + Fuc1-3 Fuc1-4 + Gal	-						[40]
N-linked Glycans	Pea	6	M5-M9	Glc	1	M4	Xyl1-2	GlcNAc	9	(+)	(+)	(+)	(+)	F1-4+Gal	[40]
N-linked Glycans	Peanut	5	M4-M8		1	M4	Xyl1-2		4	(+)	+	+	(+)	F1-4+Gal	[40]
N-linked Glycans	Pear	5	M6-M9	Glc	-				13	++	++	+	+	F1-4+Gal	[40]
N-linked Glycans	Pignoli	4	M5-M8		-				7	++	++	+	+	F1-4+Gal	[40]
N-linked Glycans	Pistachio	5	M5-M9		3	M4+M5	Xyl1-2	GlcNAc+Fuc1-3	9	++	++	++	+	F1-4+Gal	[40]
N-linked Glycans	Potato	3	M7-M9		-				7	++	++	++	+	F1-4+Gal	[40]
N-linked Glycans	Soya	6	M4-M9		-				5	(+)	(+)	(+)	(+)	F1-4+Gal	[40]
N-linked Glycans	Strawberry	4	M7-M9	Glc	1	M4	Xyl1-2	Fuc1-3	13	++	++	+	+	F1-4+Gal	[40]
N-linked Glycans	Tomato	5	M6-M9	Glc	1	M4	Xyl1-2		11	++	++	+	+	F1-4+Gal	[40]
N-linked Glycans	Walnut	5	M5-M9		2	M4	Xyl1-2	GlcNAc+Fuc1-3	14	++	++	+	+	F1-4+Gal	[40]
Cup a 1	Cupr. arizonica	-			-				3	++	++		++	F1-4+Gal	[41]

*Number of different structures.
[1]"(+)" < 10%; "++" > 65% of all examined structures (if Quant. >1 and percentages are given, or if present on all structures).
[2]R = Residue (see Figure 2).
[3]References are ordered chronologically and by allergen.
[4]"M1..M9" = Number of mannose residues.
[5]probably F1-4 + Gal = Lewis® structure [41].

Table 2. Structures of O-linked glycans presumably involved in antibody binding (non-reducing termini)

Allergen	*Source*	*Backbone*	*Linkage*	*Sidechain*	*Linkage*	*Reference*[1]
Gum arabic	Acacia senegal	Gal	β1-3	Gal	β1-6	[46]
				Glucuron	β1-4/β1-6	
				Ara	β1-2/β1-3	
				Rham	1-4	
Partly purif. Allergen	Timothy pollen	Gal	1-6	Ara	a1-3/a1-5	[47]
Ag 30 (Phl p 4)	Timothy pollen	Ara		–		[48]
Basic glycoprotein	Timothy pollen	Gal	1-3/1-6	Man	1-3/1-4	[49]
		Ara	1-3/1-5	Ara	1-3/1-5	
Phl p 1	Timothy pollen	Ara		–		[50], [51]
Extract	Mugwort pollen	Gal	1-3	Gal	1-6	[52]
		Ara	1-5	Ara	1-4	
				Glc	1-4	
Partly purif. Allergen	Clad. herbarum	Man	1-2/1-6	Gal	1-3/1-6	[53]
Ag-54 (Cla h 2)	Clad. herbarum	Man	1-2/1-6	Gal	1-2/1-3	[54]
		Glc	1-4/1-6			
H-antigen	Sea squirt	GalNAc	β1-4	Fuc	a1-3	[55], [56]
		GlcNAc	β1-3	GlcNac	β1-6	
				GalNAc	a1-2/β1-3/1-4	
Gi-rep (Gp-1β-b6)	Sea squirt	GalNAc	β1-4	Fuc	a1-3	[57], [58]
		GlcNAc	β1-3	GalNAc	a1-2	
Par h 1	Parthenium pollen	Ara		–		[59]
Extracts	7 diff. pollen	Glc	β1-3	–		[60]
Glucan	Fungi	Glc	β1-3	–		[61]
Mannoglucan	Wheat flour	Glc+Man	β1-4	–		[62]

[1]References are ordered chronologically and by allergen.

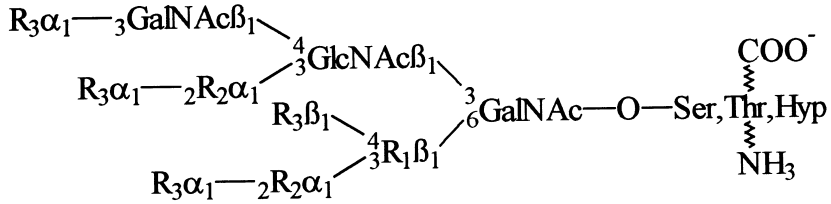

Figure 3. Structures of O-linked oligosaccharides of sea squirt allergens. R1 = –OH or GlcNAc (single case: Fuc), R2 = –OH or Fuc (two cases: GalNAc), R3 = –OH or GalNAc.

both the bromelain-type glycan (with xylβ1-2 and fucα1-3) and the fibrin-type glycan (with only mannose residues). The IgE of 91 patients (of 319 tested) reacted with the bromelain-type glycan, but only of 17 patients (of 298 tested) with the fibrin-type glycan, corresponding to 29.5% in the first and 5.7% in the second case, respectively (Table 4, unpublished data). Eighty of the patients who possessed IgE to the bromelain-type glycan were also tested with the defucosylated glycan structure carrying only the β1-2 linked xylose residue and the α1-6 linked mannose at the proximal mannose. Only 10 of these patients (12.5%) showed detectable IgE binding to this structure (Table 4, unpublished data).

These results suggest that α1-3 linked fucose is the most important residue in the epitope structure. The ubiquitous occurence and importance of this structural element was subject of many other recent studies [refs. 24,34,37,40, 70,102,121]. However, also β1-2 linked xylose was shown to be involved in a common cross-reactive antigenic determinant [63,71], not only in glycoproteins with no or little α1-3 linked fucose (this group includes for example Ara h 1 from peanut [36,37], the 48 kDa hazelnut allergen [38] and Ole e 1 [36,37]). The reason for the high immunogenicity of these two sugar residues is on the one hand that core β1,2-linked xylose residues are only present in plants or some invertebrates such as snails and schistosomes and are not present in mammalian glycans; similarly α1,3-linked fucose is also absent from mammalian glycans, while being present in plants and invertebrates such as insects and helminths. In

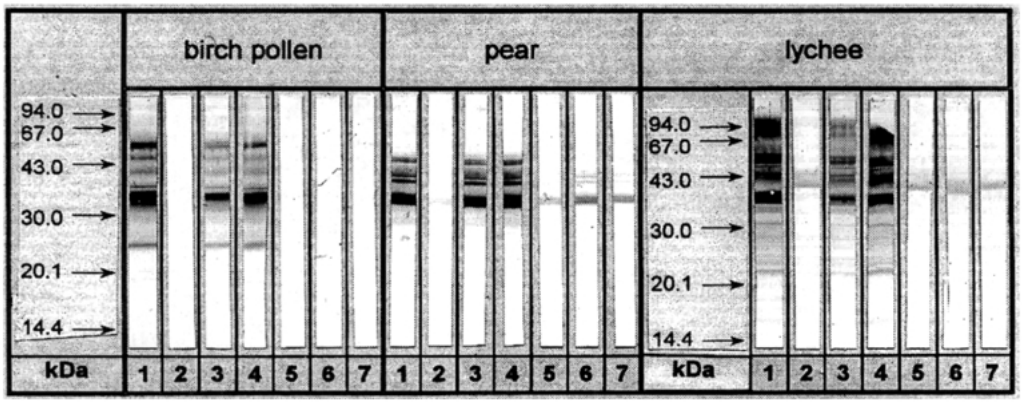

Figure 4. Detection of CCD in birch pollen-, pear- and lychee fruit extracts by immunoblot inhibition using a patient serum with a strong signal in the CCD-ELISA and a negative EAST to the 3 recombinant pear allergens Pyr c 1, Pyr c 4 and Pyr c 5. Inhibitors: 1: no inhibitor, 2: $MUXF^3$ (see Table 4), 3: MM (see Table 4), 4: rBet v 1, 5: pear extract, 6: birch pollen extract, 7: lychee fruit extract. Amount of inhibitors: Glycopeptides and recombinant allergen: 15 μg; allergen extracts: 100 μg of protein.

contrast, IgE antibodies directed against α1-6 linked fucose residues appear unlikely because these are also structural elements of mammalian glycans. This is compatible with our own results with the above mentioned carbohydrate ELISA using the immunglobulin-type glycan with α1-6 linked fucose as antigen. Only 9 of 55 tested patients (5.5%) gave a positive result with this glycan and those were also positive with the fibrin-type glycan without α1-6 linked fucose (Table 4, unpublished data).

Although from the study by Nilsen et al. [22] on Art v 2 from mugwort pollen one could conclude that the mannose residues are not involved in IgE binding, other studies indicate that they may be important for immunogenicity. Treatment with α-mannosidase showed the participation of mannose residues in the epitopes of a xylose-specific antiserum raised against carrot cell wall *β*-fructosidase [122], and of two fucose-specific antisera raised against horseradish peroxidase (HRP, [19]) and *Wistaria floribunda* lectins [123], since it resulted in losses of binding capacities of the antibodies up to 45% [123]. Furthermore, a mannose-specific antiserum raised against another purified *Wistaria floribunda* lectin [124] could distinguish between Manα1-6 substituted glycans and disubstituted glycans with an additional mannose residue in α1-3 linkage. These antibodies bound specifically to monosubstituted glycans of different lectins and of bromelain, but not to glycopeptides from fetuin and ovalbumin with additional 1-3 linked mannose residues [124]. The same observation of the importance of single sugar residues on the IgE recognition was made by van Ree et al. [37] who showed a marked difference between the binding of xylose-specific IgE antibodies to the glycans of bromelain and HRP, which differ only in the additional α1-3 linked mannose found on the HRP glycan structure (Figure 5).

This observation indicates that the anti-carbohydrate IgE might bind to more than one sugar residue of the glycan like already proposed in the model of the epitopic structure for the anti-HRP antibody [19] (Figure 6, [refs. 2,125]) and/or that the binding of anti-carbohydrate specific IgE could be sterically influenced by the presence or absence of single sugar residues [37] (Figure 5). Furthermore, some of these might act as stabilizing elements of the binding complex. Due to the small size of the carbohydrates in comparison to the antigen binding site of the antibody it seems unlikely that the antibody will only bind to a single sugar residue (Figure 6).

Results of inhibition studies using monosaccharides and monoclonal antibodies (mabs) against N-linked oligosaccharides of the glycoproteins human lgG [126] and peanut peroxidase [127] also indicate a binding of more than one sugar residue by these monoclonals. The two mabs produced against the same asialo-form of IgG recognised different parts of the glycan structure as shown by the inhibition with the single components of the glycan, but the binding of the mabs was to a certain extent specifically inhibitable by more than one sugar residue [126]. Similar results were reported for three mabs raised against the N-linked glycans of peanut peroxidase with fucose being the strongest inhibitor. The maximum inhibition achieved with 1 M fucose was only 42%, whereas inhibitions above 90% were reached with tenthousandfold less concentrated glycopeptides or glycoproteins carrying the whole glycan structure [127]. In case of a xylose-specific antiserum inhibition with xylose also failed to diminish the specific recognition of xylose-containing glycopeptides by this antiserum [122]. This indicates that even fucose and xylose are only part of the epitopic structure recognized by the anti-carbohydrate antibodies [122] or that the open forms of fucose and xylose in contrast to the ring forms as existing in the glycan structure have no or only reduced antibody reactivity. Besides, the studies of Masutani et al. [126] and Wan et al. [127] demonstrated the participation of *N*-acetyl-glucosamine in the epitopic structure of these antibodies. Among *N*-acetyl-galactosamine and fucose this sugar residue is also part of

Table 3. IgE-reactivity of N-and O-linked glycans of allergenic glycoproteins

Allergen	*Source/ Patients allergic to*	*Carbo-hydrate content (%)*	*IgE binding tested by means of*[1]	*Patients*			*Treatment of allergens*	*Effect*[2]	*CCD-part in IgE binding*	*Reference*[3]
				examined	*CCD-pos.*	*%*				
Extract	Grass+Potato +Peanut		RAST+RAST inh.	45	3	6.7	NaIO4-oxid.	RAST reduced+RAST inh. −90%	+	[67]
Extract	Derm. farinae	15	RAST inhibition (inh.)	57 (pool)			NaIO4-oxid.	RAST inh. −40%	+	[10]
Partly purif. Allergen	Timothy pollen	20	RAST inh.	30 (pool)			NaIO4-oxid.	no influence on RAST inh.	−	[47]
							Arabinofur-anosidase	no influence on RAST inh.		
Ag 30 (Phl p 4)	Timothy pollen	9.6	RAST (inh.)+RIE	Pool			NaIO4-oxid.	no influence on RAST inh.	−	[48], [72]
Partly purif. Allergen	Clad. herbarum	55	RAST inh.	18 (pool)			NaIO4-oxid.	lower IgE binding capacity	+	[53]
Ag 54 (Cla h 2)	Clad. herbarum	80.2	RAST	35 (pool)	0	0	Alkaline-borohydride TFMS	no binding to CH moieties even higher binding	−	[73]
Ara h 1	Peanut	2.4	RAST (inh.)+SPT	5 (pool)			TFMS	RAST inh. −16 to 25%	+/−	[74]
Ara h 1	Peanut		CH-RAST+Blot	66	9	13.6	Proteinase K	positive RAST results	+	[37]
PLA_2	Honeybee venom		RAST+Blot	14	11	78.6	Pronase	RAST inh. up to 100%	+	[75]
			Use of unglycosylated variant	14	11	78.6		reduced or no IgE binding		
PLA_2	Honeybee venom		CH-ELISA+Blot	47	34	72.3		18 patients: CH + protein epitopes	+	[24]
			Use of non-glycosylated variant					16 patients: only CH epitopes		
PLA_2	Honeybee venom		T cell+PBMC proliferation	3	2		Endo F/N-glycosi-dase F	No proliferation	+	[76]
			Use of non-glycosylated variant	3	2			No proliferation		
Xan VIa	Xanthium strumarium	18	RAST (inh.)+SPT	6 (pool)			NaIO4-oxid.	RAST inh. −40%	+	[77]
Extract	Caddis fly		RAST (inh.)+Blot	13	3	23.1	NaIO4-oxid.	RAST inh. −50 to 80%	+	[69]
Gi-rep	Sea squirt		Use of oligosaccharide in SPT	20	20	100	NaIO4-oxid.	no skin reaction	+	[78]
							Pronase E	nearly no influence on skin reaction		
H-Antigen	Sea squirt		Use of oligosaccharide in SPT	8	8	100		6 of 11 structures SPT-positive	+	[55]
Gi-rep (Gp-2)	Sea squirt		SPT	6	6	100	NaIO4-oxid.	no skin reaction	+	[57]
Gi-rep (Gp-1β-b6)	Sea squirt		Use of oligosaccharide in SPT	6	6	100	β-HexNAcase	no skin reaction	+	[58]
				6	6	100	a-GalNAcase	no skin reaction		
Extract	Altemaria	66	ELISA inhibition+SPT	Pool			TFMS	Allergen activity eliminated	+	[79]
Alt a I_{1583}	Altemaria altemata	20	ELISA+Blot	ng			TFMS	IgE binding abolished	+	[80]
Par j 1	Parietaria		ELISA+Blot	Pool			TFMS	3-fold reduction of ELISA inh., but secondary structure changed!	?	[81]

Extract	Parietaria		ELISA+Blot	Pool			TFMS	no influence on ELISA inh.	−	[82]
Art v 2	Mugwort		ELISA+Blot	Pool	0	0	PNGAse F	No difference to denatured allergen	−	[22]
Fel d 1	Cat	20	RIA+SPT+Blot	Pool			N-Glycanase	2 to 4-fold reduction of RAST inh.	+	[83]
Fel d 1	Cat		RIA (inh.)	6 (pool)			TFMS	RIA −85%, RIA inh. −90% but secondary structure lost!	?	[84]
							NaIO4-oxid.	6-fold reduction of RIA inh.	+	
							Endo F/N-glycanase	2-fold reduction of RIA inh.		
Cry j 1	Cryptomeria		ELISA+SPT	27*	2		Protease	only glycos. Fragments bound	+	[85]
Extracts+CH	Pollen+veg. Food		CH-ELISA (inh.)+Blot	54	12	22.2	NaIO4-oxid.	Blotbands quenched ELISA inh. −80 to 100%	+	[86]
Extracts	Asp. fumigatus	8.3–33.7	RAST+Blot	1			NaIO4-oxid.	38+90 kDa Blotband quenched	+	[87]
Ole e 1	Olive pollen	5	ELISA inh.+Blot	Pool			TFMS	17-fold reduction of ELISA inh., but secondary structure lost!	?	[88]
							PNGAse F	10-fold reduction of ELISA inh.	+	
Ole e 1	Olive pollen		ELISA+Blot	10 (pool)			NaIO4-oxid.	Xylβ1-2 involved in IgE binding	+	[89]
Ole e 1	Olive pollen		Dot blot+BHR	23	15	65.2	NaIO4-oxid.	decreased or no IgE binding	+	[90]
				5*	5		Free CH used in BHR	40 to 60% BHR with 5 Patients		
Ole e 1	Olive pollen		CH-RAST+Blot	66	34	51.5	Proteinase K	positive RAST results	+	[37]
Phl p 1	Timothy pollen	1.7–6.8	(2D) Blot+Lectin binding	ng	1		Glycosidases	Deglycosylation incomplete		[50]
				2*	1		TFMS	Blotbands quenched	+	
				3*	1		NaIO4-oxid.	Blotbands quenched		
Phl p 1	Timothy pollen		IEF-print+ELISA+Blot	14*	1		NaIO4-oxid.	Blotbands quenched	+	[91]
			Use of recombinant protein	14*	1			rPhi p 1 not recognized		
Phl p 1	Timothy pollen		ELISA+Dot blot	274	58	21.2	TFMS	57 patients: CH + protein epitopes	+	[92]
			Use of defined glycoproteins					1 Patient: only CH eptiopes		
Extracts	Grass pollen+Fruit		CH-RAST+Blot + SPT+BHR	11	10	90.9	Proteinase K	positive RAST results	+	[93]
				1*	1		inh. with digested extract	RAST inh. more than 90%		
Lol p 11	Rye grass	8	RAST (inh.)	165	64	38.8	Proteinase K	positive RAST results	+	[94]
				2*	1		TFMS	RAST inh. abolished		
Lol p 11	Rye grass		CH-RAST+Blot	66	37	56.1	Proteinase K	positive RAST results	+	[37]
Par h 1	Parthenium pollen	40	ELISA+Blot	6 (pool)			Pronase E	only 25% binding activity lost	+	[59]

Table 3. (*continued*)

Allergen	Source/ Patients allergic to	Carbo-hydrate content (%)	IgE binding tested by means of[1]	Patients examined	Patients CCD-pos.	Patients %	Treatment of allergens	Effect[2]	CCD-part in IgE binding	Reference[3]
							NaIO4-oxid.	drastic reduction of allergenicity		
							TFMS	loss in IgE binding capacity		
							Glycanases	no influence on IgE binding		
Extract	Tomato		EAST+CH-ELISA+Blot	8	7		NaIO4-oxid.	Blotbands quenched	+	[70]
Extract	Tomato		EAST+CH-ELISA+Blot	32	12	37.5	Inh. with glycans	Blotbands quenched	+	[95]
							Inh. with glycans	EAST inh. up to 84%		
BMAI-1 + WTAI-CM16	Barley+Wheat flour		RAST+Blot+Lectin	5 (pool)			TFMS PNGAse A	Blotbands quenched Deglycosylation incomplete	+	[71]
Extract	Horse dander		Blot+Lectin binding	36*	1		NaIO4-oxid.	14.4 kDa Blotband quenched	+	[96]
BG 60	Bermuda grass	7.5	ELISA+Blot+SPT	18*	>=1 (ng)		NaIO4-oxid.	ELISA-65%	+	[32]
Extract	Latex		CAP+Blot	56*	4		NaIO4-oxid.	30–60 kDa Blotbands quenched	+	[97]
Extract	Kiwi		ELISA+Blot	7	5		NaIO4-oxid.	25–90% inh. of IgE binding	+	[98]
				5	4		Inh. with Prot.K-Extract	ELISA—30 to 70%		
Parvalbumin	6 Fish species		RAST+Blot	30*	2		NaIO4-oxid.	12 kDa Blotband quenched	+	[99]
Gum arabic	Acacia senegai	98	CAP+EAST+Blot+SPT	1	1		NaIO4-oxid.	no EAST inhibition Blotbands quenched	+	[100]
Extract	Celery		EAST+CH-ELISA+Blot	30	9	30	Heat treatment	no influence on CCD binding	+	[101]
Extract	Celery		EAST+CH-ELISA+Blot	10 1* 1*	8 1 1	80	Inh. with glycans	EAST inh. up to 100% Blotbands quenched 25% BHR with 1 Patient	+	[102]
Extract	Celery		CAP+EAST+Blot	22	12	55	Inh. with glycans	EAST inh. up to 100% Blotbands quenched	+	[103]
Extract	Pear		CH-ELISA	61	10	16.4	Inh. with glycans	Blotbands quenched	+	[104]
Mannan	Pitysporium ovale		Mannan ELISA	4 (pool)					+	[105]
Mannan	Candida albicans		Mannan ELISA+RAST PBMC proliferation	15	15	100		10-fold stronger proliferation with mannan than with protein	+	[106]
Extract	Cupressus arizonica	74	CH-ELISA+Blot	15	15	100	NaIO4-oxid.	CH-ELISA inhibition −65% Blotbands quenched	+	[107]
Ascorbate Oxidase[4]	Cupressus arizonica		ELISA	10	10	100	NaIO4-oxid.	99% reduction of IgE binding	+	[39]
Polyamine Oxidase[5]	Cupressus arizonica		ELISA	10	10	100	NaIO4-oxid.	99% reduction of IgE binding	+	[39]

Extract	Zucchini	CAP+EAST+Blot + SPT	4	2	50	Inh. with glycans	EAST inhibition up to 60% Blotbands quenched	+	[108]
Hev b 2	Latex	ELISA+Blot	ng			NaIO4-oxid.	loss in IgE binding capacity reduced binding	+	[109]
		Use of non-glycosylated variant	ng						
Gly m Bd 28k	Soybean	Blot+Dot blot	7	7		Glycopeptidase A	no recognition of deglyc. peptide	+	[110]
			7	5		Inh. with anti-HRP mAb	reduced IgE binding		
CH+Extracts	Pollinosis	Bromelain CAP	326	136	41.7			+	[111]
		CAP+ELISA+Blot + SPT	1*	1		NaIO4-oxid.	CH-ELISA—50 to 90%		
Extract	Hazelnut	Blot+RBL test	12	5	41.7	Inh. with glycans	Blotbands quenched	+	[38]
			28	17	60.7				
Le^a-BSA+Le^x-BSA	Tree+grass pollen	CH-RAST	31	0	0			−	[37]
Mannnoglucan	Wheat flour	ELISA inh.	4	4			20 to 90% inh. with polysacch.	+	[62]
Extracts	Hymenoptera venoms	RAST/CAP+Blot	15	11	73	NaIO4-oxid.	Hyaluronidase band quenched	+	[112]
Extracts	Hymenoptera venoms	RAST/CAP (inh.)	16	9	56.3	Inh. with Bromelain-BSA	complete Inh. of IgE binding	+	[113]
Fes p 4	Festuca pratensis	ELISA inh.+Blot	8	6	75	NaIO4-oxid.	reduced IgE binding	+	[114]
Extract	Carrot	CAP+EAST+Blot + DBPCFC	26	11	42.3	Inh. with glycans	Blotbands quenched	+	[115]
Extract	Persimmon	CAP+EAST+Blot +DPCFC	3	3	100	Inh. with glycans	EAST inh. up to 90% Blotbands quenched	+	[116]
Bromelain+PHA-L	Veg. Foods	CH-RAST+SPT+ICT	55	9	16.4			+	[117]

[1]inh. = inhibition; CH = carbohydrate.
[2]Referring to the CCD-pos. Patients (if these are indicated) and calculated from figures (if percentages are not given from the authors).
[3]References are ordered chronologically and by allergen.
[4]From Cucurbita pepo, used as model allergen with patients allergic to Cupressus pollen.
[5]From maize, used as model allergen with patients allergic to Cupressus pollen.
*Not all patients tested for CCD-positivity.
ng = not given.

Table 4. IgE reactivity of pollen allergic patients to CCD structures, tested by carbohydrate ELISA

N-glycan of	*Structure*	*Short term*	*% positive patients*
Bromelain	Manα1-6(Xylβ1-2)Manβ1-4GlcNAcβ1-4(Fucα1-3)GlcNAc	MUXF[3]	29.5
Brom., defuc.*	Manα1-6(Xylβ1-2)Manβ1-4GlcNAcβ1-4GlcNAc	MUX	12.5
human IgG	Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAc	MMF[6]	5.5
Fibrin	Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNac	MM	5.7

*Brom., defuc. = Bromelain, defucosylated.

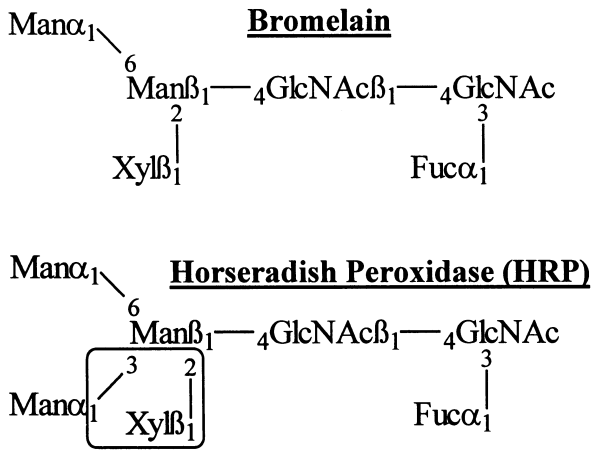

Figure 5. N-linked glycans of bromelain (MUXF[3]) and horseradish peroxidase (HRP). The potentially sterical hindrance of xylose-specific IgE antibodies to HRP by the additional mannose in 1,3-linkage is depicted by a box (according to [37]).

some of the disaccharide units proven to be the allergenic epitopes of the sea squirt allergens H-antigen [55,56] and Gi-rep [57,58].

The characterization of biologically active disaccharide units in case of the sea squirt allergens indicated that on these allergenic glycoproteins also at least two sugar residues were involved in IgE binding.

Initial results obtained with the newly discovered Lewis A determinants of allergenic plant extracts coupled to BSA showed that these widespread structures [15,35,40] which play an important role in cell-to-cell recognition of mammalian cells fail to induce an IgE response in tree and grass pollen allergic patients [37].

Prevalence of IgE to CCD in different populations

The prevalence of CCD-positive patients is in many cases not calculable, since several studies used pool sera, tested only selected sera from the whole patient panel for CCD reactivity or even had only single patients at their disposal. Furthermore, it depends on the allergen and on the preselection of the patients. Therefore frequencies from 0% to nearly 100% have been reported (see Table 3). If larger numbers of patients (>100 if available) were included then the prevalence of anti-glycan IgE varied between 20 and 40% for grass pollen allergics [92,94,128,129]. This corresponds with own observations in patients allergic to tree and grass pollen with around 30% of the patients' IgE recognizing the bromelain glycan (see above and Table 4). Higher frequencies of IgE reactivity were reported in studies in olive pollen (51–65%; [37,90]) and in cupressus pollen positive patients (100%; [39,107]).

Patients with confirmed food allergies have prevalences of IgE to CCD in the same range as grass and tree pollen allergic patients. So, sensitizations to glycans of pear [104], tomato [95], carrot [115], hazelnut [38] and celery [101,103] occur in 16 to 55% of the patients allergic to the foods. Higher incidences of CCD sensitization (56–79%) are to be found among patients allergic to Hymenoptera venoms [24,75,112, 113] and among the patients allergic to sea squirt allergens with 100% CCD reactivity [55,57,58]. This indicates that some allergens (e.g. from cupressus pollen extract and from sea squirt) might influence the production of anti-carbohydrate IgE by their high content of immunogenic carbohydrates.

Clinical relevance of the oligosaccharides linked to allergenic glycoproteins

Beside others functions, carbohydrates protect glycoproteins against thermal denaturation and enzymatic digestion. This suggests that they are indirectly, but also directly, during the protein biosynthesis, involved in the formation and conservation of the secondary structure of the protein by steering the folding of the polypeptide chain. Since the secondary structure determines the conformation of the surface and core structure, the glycan moieties fulfill an important function for the conservation of the biologic activity and IgE binding capacity of the glycoproteins.

As mentioned earlier in the text, a prerequisite for causing mediator release, and thereby eliciting allergic symptoms, is the presence of a polyvalent antigen that is able to cross-link cell bound IgE on mast cells and basophils. The cross-linking could occur either by CCD specific antibodies, if more than one glycan is present on the surface of the glycoprotein (Figure 1C), or by combinations of CCD specific and peptide specific antibodies (Figure 1B). In the recent years some authors concluded that IgE antibodies to CCD are biologically inactive

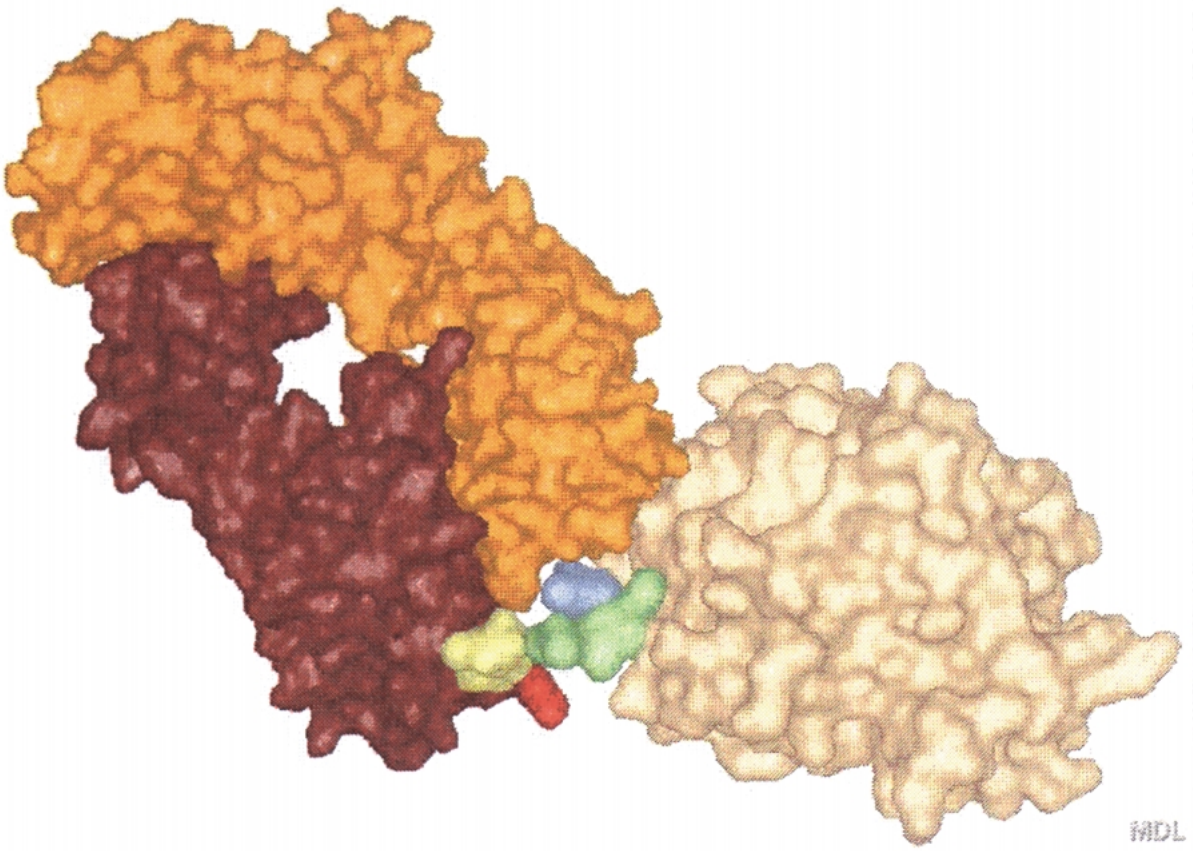

Figure 6. Proposed model of the epitopic structure for the anti-HRP antibody [19] using the theoretical model of a Fab fragment complexed with a ganglioside-type carbohydrate (PDB-No. 2PSK, [125]) by replacing this carbohydrate with the HRP-type glycan of the *Erythrina corallodendron* lectin (PDB-No. 1AX0, [2]). The Fab fragment is depicted by the brown and orange chains. The sugar residues of the HRP-type glycan that is bound to Asn17 of the lectin (lower right corner) are individually coloured. The binding relevant fucose and mannose residues [19] are depicted in blue and yellow, respectively. The figure was prepared with Protein Explorer and afterwards processed with CorelPHOTO-PAINT!^{TM}.

and therefore clinically irrelevant [111,128–132], or that the interaction of anti-CCD IgE with the glycans has too low affinity for effective cross-linking [132]. However, the tests to prove these hypotheses were either not performed until now or were insufficient for convincing conclusions. For instance, van der Veen et al. [131] investigated a panel of patients who are IgE positive but non-allergic to peanut, showing that anti-CCD IgE in these patients had no or only poor biologic activity. Yet no other results would have been expected, since this patient group was non-allergic. In 1999, Marl et al. [111] tested 136 bromelain-CAP positive patients with pollinosis by skin test with a bromelain solution and none of them showed a positive skin test response. This result is also not surprising since bromelain carries only one carbohydrate chain and is therefore incapable of inducing a histamine release by cross-linking of carbohydrate-specific IgE antibodies (for example see Figure 1A). This is truly the case for many other glycoproteins with only mononvalent glycans, although these might act as additional cross-linking elements beside the peptidic epitopes (Figure 1B). The last fact could explain the histamine release results of 5 patients with Ole e 1, which possesses only one N-linked glycan, but not the strong mediator release with the purified free carbohydrates of Ole e 1 [90]. Although the authors believe that the CCD structure might be large enough (15 A) for the cross-linking of IgE antibodies on the surface of mast cells and basophils, this is, in our opinion not very likely (see also Figure 6). Also, these data have so far not been confirmed by other researchers.

In our study [102] a celery-allergic and CCD positive patient showed no histamine release with the purified glycans of bromelain and fibrin and with BSA, but up to 25% mediator release with BSA-conjugates of the bromelain glycan (3 to 4 mol per mol protein). This result indicates the necessity of polyvalent epitopes for the biologic activity of the glycoproteins. Besides, we found single celery-allergic patients whose only detectable IgE directed against celery was specific for CCD without any additional recognition of protein allergens of celery [102,103]. These are further clues that CCD may be clinically relevant at least in a subgroup of IgE-positive patients.

Concluding remarks

Many studies have shown without any doubt that N- and O-linked oligosaccharides are important epitopes for human IgE. It is evident that N-glycans with α1-3 linked fucose and β1-2 linked xylose, or with fucose alone are the most important elicitors of IgE-responses in allergic patients. In parallel to the development of refined methods of glycoprotein analysis, sophisticated methods to determine anti-carbohydrate IgE responses and for studying epitope structures have been developed. However, the question of the clinical relevance of these antibody responses is still open. Only in the case of the O- and N-linked oligosaccharides of the sea squirt allergens H-antigen and Gi-rep it was clearly shown by skin testing that these carbohydrate moieties are involved in the triggering of skin and conjunctival symptoms in sea squirt-allergic patients [55,57,58]. By contrast, none of the studies in plant food allergy revealed a conclusive answer to this question. In this area, carefully designed studies are required to end the theoretical discussion about the clinical significance of the carbohydrate parts of the glycoproteins. This could be achieved by skin test or histamine release studies with pure IgE-binding glycoproteins and their non-glycosylated counterparts in patients with confirmed allergies to certain plant foods or pollen, in comparison to IgE-positive control groups without allergic symptoms. Neoglycoproteins conjugated with multiple units of single glycans could serve as helpful tools in such studies which are underway in several laboratories. Yet there is still one important point to consider: the specific sugar recognition patterns of the anti-carbohydrate antibodies [37,121,124,126,127] indicate that minor structural differences of the glycans could be of great importance for the IgE recognition and cross-linking (see also Table 4). Recent results concerning the profiles of N-linked glycans of many plant extracts showed a very different distribution pattern of the glycans [34,40]. That is why, it seems not enough to use only polyvalent glycoproteins such as HRP for all patients regardless of the origin of their allergic diseases, but that these glycans or glycoproteins also have to share structures actually present in the allergenic extracts. Moreover, in many cases more than one glycan have to be tested for biological reactivity because in most extracts several different glycans showed prominent abundances [34,40] and could therefore be clinically relevant for the CCD-positive patient group. The importance of the source of the glycan used for testing was also underlined by a recent review [133].

Acknowledgments

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