

IGE-REACTIVE CARBOHYDRATE EPITOPES - CLASSIFICATION, CROSS-REACTIVITY, AND CLINICAL IMPACT

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SUMMARY: A glycan-related IgE-reactivity has been demonstrated in most allergen sources, especially in plant kingdom. Recent progress in glycobiology has allowed a clearer classification of these glyco-epitopes. Unlike classical peptide chain-based epitopes, glyco-epitopes can share significant structural homologies beyond the limits of protein families. These glyco-epitopes are thus prone to extensive cross-reactivity. They have been called Cross-reactive Carbohydrate Determinants or CCD. Many of these glyco-epitopes behave as " panepitopes ", leading to cross-reactivity between products as distant as pollens and hymenoptera venoms. But CCD are not universally cross-reactive and they rather cluster into subgroups such as plant CCD or fungal CCD. Because a monovalent IgE-binding is sufficient in serum-based assays, glyco-epitopes and CCD are classically considered as a potential source of positive *in vitro* results without clinical significance. But some authors recently demonstrated that glyco-epitopes could induce a response at the cell level and suggested that they might play a role *in vivo*. As long as *in vitro* assays include glycan- and CCD-related IgE responses, laboratory results should be carefully interpreted in the light of existing knowledge about the glycomes of natural products. IgE-reactivity of the patient's serum can also be tested towards a glycoprotein model such as bromelain.

Key-words: glycoproteins, epitopes, cross-reactions, CCD, specific IgE, allergy diagnosis

INTRODUCTION

In 1999, Mari *et al.* described the case of a 36-years old patient, living in Rome, who consulted for a seasonal rhino-conjunctivitis [1]. Skin testing confirmed a sensitization to grass and juniper pollens. *In vitro* assays were found positive for these pollens but, unexpectedly, to many other products such as birch, mugwort, and olive pollens, but also cat, honeybee, cockroach, and even latex. These apparent false results were confirmed with different commercial reagents and on other occasions during the patient's follow-up.

As shown by these authors, these unexpected data resulted from the simultaneous presence of cross-reactive carbohydrate determinants (CCD) in the allergen extracts and of anti-CCD IgE in the patient's serum.

That carbohydrate structures could contribute to allergic sensitizations had been suspected for a long time, even before IgE characterization (2). Aalberse played an instrumental role in underlining the importance of these glyco-epitopes in allergy diagnosis, and he coined the term of CCD (3).

Since then, many points have been better understood: one knows that anti-CCD IgE are often found in the serum of pollen allergic patients, that many plant allergens are glycoproteins and, as such, potent sources of CCD, and that carbohydrate epitopes are able to give rise to interferences in specific IgE assays for latex and for hymenoptera venoms (4, 5).

This article aims at reviewing our present knowledge about CCD: the biochemistry and classification of carbohydrate structures on glycoproteins, the reasons why they can give cross-reactions, the occurrence of CCD in allergenic products, the extent of the cross-reactivity due to CCD, and finally the arguments for and against a clinical impact of CCD.

BIOCHEMISTRY AND ALLERGENICITY OF CARBOHYDRATE STRUCTURES (6)

Glycoproteins

Protein synthesis does not end with mRNA transcription: as soon as the peptide chain is available, complex and finely regulated mechanisms come into play to give a correct folding to the final protein: cleavage of

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a pro-peptide, coupling cysteines into disulfide bridges, addition of carbohydrate chains, etc. This last event gives rise to a glycoprotein.

Among proteins, many are glycoproteins. In addition to a correct folding, glycosylation confers them some important features such as a better hydrophilicity and a stronger resistance to thermic shocks (7, 8). Carbohydrate chains borne by membrane proteins play a protective role and participate into the cell signature (eg. blood groups).

Glycan chains (9-11)

Carbohydrate chains, or glycans, are fixed onto the peptide at specific sites. One distinguishes N-linked glycans and O-linked glycans, depending on the amino acid the glycan is attached to: an asparagine for N-glycans, and a serine, a threonine, or an hydroxyproline for O-glycans. Most glycoproteins are either O-glycated or N-glycated.

Although functionally important, glycans often account for a minor part of the protein weight (eg. less than 5%). Figure 1 shows an example of N-glycan rather common in plant proteins. Sometimes, carbohydrates represent a larger content (eg. 30% in mugwort allergen Art v 1) (12) and even a dominant part of the glycoprotein (eg. mannoglucans found in gums and moulds) (13, 14).

From glycans to epitopes

Most often, carbohydrate structures are prominent and thus can easily constitute an immunogenic site (T epitope) and/or an IgE binding site (B epitope) (see figure 2).

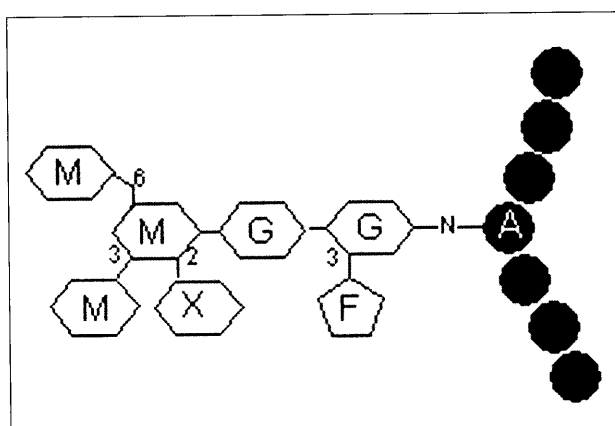


Figure 1: An example of N-glycan chain.

The 7-sugar chain is linked to the peptide on an asparagine (A) and is composed of two N-acetylglucosamines (G), three mannose (M), one 1,3-fucose (F) and one 1,2-xylose (X). This "MMXF3" chain is often found in plant glycoproteins.

One does not know what is the minimal length for a glycan to act as a B-epitope, but most carbohydrate epitopes characterized so far comprise at least 4 sugars.

Are carbohydrate epitopes restricted to the glycan chain? Or could macromolecular flexibility give room to a role of adjacent amino acids in IgE binding? (16). The question is still debated. But regarding IgE molecules themselves, some flexibility is indeed possible at the paratope site and this results in an imperfect specificity of a given IgE molecule towards a unique allergen epitope (17). In that respect, carbohydrate epitopes do not differ from peptide epitopes: they are able to bind to more than one "specific" IgE (18), and these cross-reactive epitopes are thus named CCD.

Glycan diversity

Glycan structures are at least as diverse as peptide sequences: each sugar enables several possible linkages with the following sugar. More than 1000 spatially different combinations can be deduced from a chain comprising only 3 sugars.

Cells take advantage of this diversity to meet their present metabolic or defence needs through finely modified glycoprotein structures. Indeed, natural glycoproteins most often appear as a mix of variants (eg. as many as 16 different glycan combinations on honeybee allergen Api m 1) (19).

This microheterogeneity gives rise to imprecise bands on immunoblots, especially when molecular weights exceed 30-40 kDa. Another diversity consists in the simultaneous presence of glycosylated and non-glycosylated isoforms resulting in double bands (eg. Ole e 1 in olive pollen (20) or Pla I 1 in English plantain) (21).



Figure 2: An example of glycoprotein.

This mannosidase from *S. cerevisiae* presents three glycan chains at its outer surface. One of them has 7 sugars and could become an epitope. Protein Data Bank ID = 1DL2 (15).

Cross-reactivity in spite of diversity

One should expect that such a structural diversity would limit cross-reactivity between glycan chains. In fact, IgE-reactivity is greatly driven by the presence of some sugars at specific positions along the chain. And certain basic sugar patterns are indeed found among IgE-reactive glycan chains, especially N-glycan ones (figure 3). For all eukaryotes, N-glycan synthesis starts with the addition of the same 7-sugar chain. Then, this basic chain is elongated and/or trimmed. Xylose is a characteristic sugar of IgE-reactive plant N-glycans. A fucose is often found too, but a 1,3 spatial setting seems critical to its IgE-reactivity [6, 22]. Such 1,3-fucoses are a frequent finding in plant and some invertebrate N-glycan chains and this feature leads to potential cross-reactivity (eg. between pollen and hymenoptera allergens). Mammal N-glycans are 1,6-fucosylated and thus do not cross-react with 1,3-fucose N-glycans (see figure 3).

One could expect a similar tendency to cross-reactivity among O-glycan chains. But, except for some allergens such as mugwort pollen Art v 1 [23], this kind of glycans has been less well studied than N-glycans and the frequency of O-glycan-based CCD is largely unknown.

Glycomes: the complexity of natural products

Natural products, allergenic or not, contain a large number of different proteins. Among these proteins, some are glycoproteins. Among glycoproteins, some

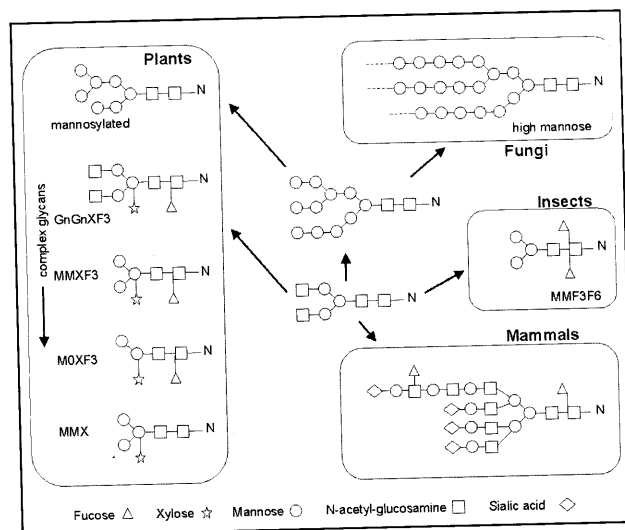


Figure 3: Diversification of N-glycans among eukaryotes

The process starts with the addition of a 7-sugar chains at the N-glycosylation site (an asparagine). This oligosaccharide, common to all eukaryotes, is then modified by trimming and adding sugars. These modifications lead to a diversification of N-glycans along the phylogenetic branches of eukaryotes. A notation is used to describe the glycans, especially the presence and the position of mannose (M), xylose (X), fucose (F), and N-acetyl glucosamine + mannose (Gn).

are known allergens. Among the epitopes of these allergens, some consist in carbohydrate chains. And among these glyco-epitopes, certain are prone to cross-react with glycan chains present in other allergens, ie. to behave as CCD.

Any natural allergenic product is thus susceptible to contain allergens and non-allergens, peptide- and/or glyco-epitopes on each allergen molecule, and cross-reactive or not cross-reactive epitopes. The response observed during in vitro assays, as well as in clinical reactions, is thus global.

Facing to such complexity, some help can however be found in "glycomes". A glycome corresponds to the percentage of all types of glycan chains present in the extract. For example, grass pollens are rich in M0XF3 glycans (see fig. 3), and peanuts contain a noticeable proportion of high-mannose chains (24, 25). This kind of information can give a useful clue in some situations of suspected cross-reactivity.

HOW CAN CCD BE DEMONSTRATED?

First of all, one need to prove the existence of glycan chains onto the protein. To this end, one can use the property of natural lectins, (eg. concanavalin A) to bind to specific sugars (25, 26). More precise methods consist in monoclonal antibodies (25, 27) or mass spectrometry (eg. MALDI-MS-MS) (28).

Then one must demonstrate that these glycans are able to bind to IgE. Patients sera are therefore used. To differentiate what is related to peptide epitopes from what concerns glyco-epitopes, different methods are available:

- one can cut up the allergen with a proteinase K: any remaining IgE reactivity signs the presence of carbohydrate epitopes;
 - one can cut out glycans from the peptide backbone with a PGNase: any loss of IgE reactivity means that glyco-epitopes did play a role before;
 - the same reasoning applies to periodate treatment of the extract, as this chemical oxidation modifies glycan structures;
 - finally, one can compare the response of a natural allergen with that of its non-glycosylated recombinant version (eg. a recombinant expressed in *E. coli*) (29).
- The next step consists in checking that the newly demonstrated glycan shows cross-reactivity with other glycoproteins, ie. it can be labelled as a CCD. As for peptide epitopes, cross-reactivity between glyco-epitopes is commonly studied by using inhibition methods. Two main sources of inhibitors are currently applied:
- bromelain, a glycosylated allergen extracted from pineapple; this inhibitor is widely used because its M0XF3 carbohydrate chain is also found in many

plant proteins and because a true allergy to bromelain is very rare (30, 31);

- glycopeptides obtained after a peptide cleavage deep enough to ensure that peptide epitopes are no longer present (32-34).

OCCURRENCE OF CARBOHYDRATE EPITOPES AND CCD IN NATURAL PRODUCTS

Knowing which glycan chains are present on which allergens is important to better assess the possible impact of glyco-epitopes and CCD on diagnostic test results

Plant-derived products

Many plant proteins are glycosylated. Figure 4 shows the main families of allergens found in plant natural products. Glycoproteins are obviously more frequent among proteins whose molecular mass is 30 kDa or heavier. A glycan-related IgE-reactivity has been demonstrated for most of these glycosylated allergens. But such IgE-reactivity does not imply an ever-present cross-reactivity between plant glycoproteins.

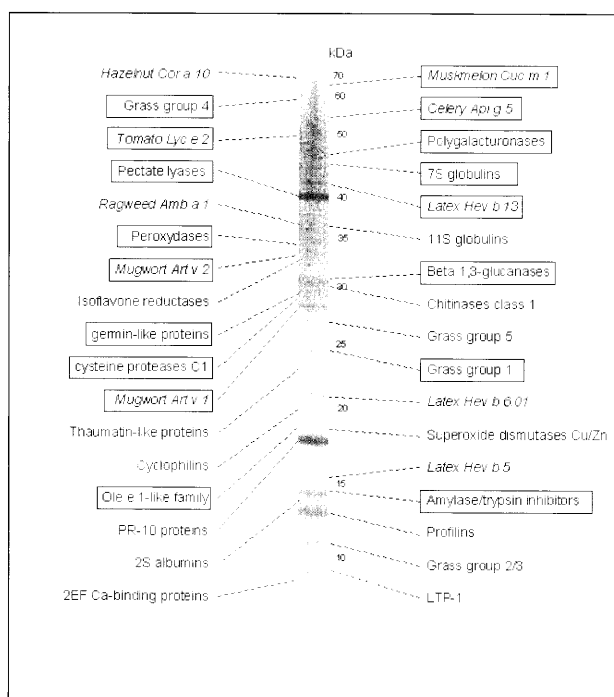


Figure 4: Glycosylation of main plant allergens and allergen families

Glycosylated allergens (in *italics*) and glycosylated allergen families are shown in a box. For some families, these properties are not systematic : for example, a few 2S-albumins and thaumatin-like proteins are glycosylated and some cysteine proteases C1 are not. Average molecular weights (MW) are given along an artificial immunoblot.

A minimal homology between glycan chains is required to make a carbohydrate epitope a CCD.

Glycosylated allergens (in *italics*) and glycosylated allergen families are shown in a box. For some families, these properties are not systematic : for example, a few 2S-albumins and thaumatin-like proteins are glycosylated and some cysteine proteases C1 are not. Average molecular weights (MW) are given along an artificial immunoblot.

Pollens

Pollens represent the best studied case of IgE-reactivity to glyco-epitopes. The prevalence of anti-glycan IgE in the serum of pollen allergic patients has been estimated with two indirect *in vitro* methods: the positivity to CAP Pharmacia bromelain test and the residual response to proteinase-K-treated pollen extracts. Bromelain positivity rates as high as 23-42% were observed in Italian pollen allergic patients (1, 31). Similar rates were found in Belgium (23%) (35), in Germany (29%) (6) or in Spain (52%) (36). Highest rates of glycan-related IgE-reactivity were found in patients sensitized to several pollens (22, 31). This was confirmed by Ebo (37) who found positive bromelain tests in birch allergic patients only if these patients were also allergic to another pollen. Furthermore, 21 out of the 23 pollens tested by Mari cross-reacted with bromelain (38).

With proteinase-K-treated extracts, anti-CCD IgE were found to be present in Dutch pollinic patients at rates as high as 51% for mugwort, 56% for orchard grass, and 83% for birch (39). The same team observed that most Spanish pollinic patients living in a birch-free area were nonetheless positive to a proteinase-K-treated birch extract and thus had anti-CCD IgE (40).

The existence of anti-CCD IgE among pollen allergic patients seems established, but one does not clearly know if some pollens are especially efficient for sensitizing the patient to glycan chains. Solving this question is complicated by the scarcity of mono-pollinic patients: for example, most subjects sensitized to olive pollen are also sensitized to grass pollens; and the same applies to mugwort and birch and/or grass pollens (41). Moreover, the presence of glyco-epitopes in some important pollens has been poorly studied: for example, CCD are only suggested in ragweed (42).

➤ **Grass pollens:** Most of our knowledge about CCD in pollens comes from works on grass allergens. These plant pollens are especially rich in glycoproteins: group 1 (beta expansins such as Phl p 1, Lol p 1,...), group 4 (Phl p 4, Fes p 4,...), group 11 (Lol p 11 and other Ole e 1-homologues), group 13 (polygalacturonases), and BG-60, a Bermuda grass allergen close to Phl p 4, all are glycosylated (43). And a gly-

can-related IgE-reactivity could be confirmed for group 1 allergens (44, 45), for group 4 (46, 47), for Lol p 11 (39), for Phl p 13 (45), and for BG-60 (48). Grass pollens thus seem an especially powerful source of anti-glycan IgE.

➤ **Tree pollens:** Oleaceae pollens contain allergens homolog to Ole e 1, the main olive pollen allergen: like Ole e 1, lilac Syr v 1, ash Fra e 1, and privet Lig v 1 are glycosylated. A glycan-related IgE-reactivity has been demonstrated for Ole e 1 (33, 39), for Fra e 1 (49), and for Ole e 9, an olive beta-1,3 glucanase allergen (50); it was suspected in high molecular weight (HMW) proteins of ash tree pollen (51).

Main allergens from Cupressaceae pollens are glycoproteins: they belong to the pectate lyase family (Japanese cedar Cry j 1, Arizona cypress Cup a 1,...) and to the polygalacturonase family (Cry j 2, juniper Jun a 2,...) (52). It has been shown that Cup a 1 could behave as a glycan-reactive allergen (53). And the cross-reactivity between tomato and Japanese cedar is affected by periodate (54). On the other hand, the contribution of glycans in Cry j 1 IgE-reactivity seems low (55, 56).

Another polygalacturonase, Pla a 2, has been recently characterized as a major allergen in the pollen of plane tree (57). The IgE-reactivity of Pla a 2 glycans is unknown, as well as that of grass or Cupressaceae polygalacturonases.

In birch pollen, the main allergens Bet v 1 and Bet v 2 are not glycosylated. One thus could expect no anti-CCD IgE in the serum of patients suffering from birch pollinosis (and maybe from Fagales pollinosis at large). Indeed, Ebo *et al.* found positive bromelain tests in birch allergic patients only if these patients were also sensitized to grass pollens (37).

A relation between birch pollen and CCD is probable, however. Birch IgE-reactivity is affected by periodate (58). And a birch extract could inhibit 30-80 kDa bands on immunoblots of many plant foods (59-66). Owing to the frequency of glycoproteins among HMW proteins, these results give credit to the presence of CCD-bearing proteins in birch pollen. Possible candidates could be a Phl p 4-like allergen recently characterized in birch (67) and/or Bet v 8, a 65 kDa pectinesterase (68).

➤ **Weed pollens:**

At least two mugwort pollen allergens are glycosylated:

- Art v 1 (27-29 kDa) presents a long O-glycan chain made of galactose and arabinose (23). This kind of O-glycan is also found in Par h 1, an allergen of the tropical weed *Parthenium* (69), and is slightly different from glycans found in gums [13] or in Solanaceae lectins and extensins (70). The O-glycan chain of Art v 1 was shown to be IgE-reactive (23).
- Art v 2 is also unusual: it bears a N-glycan chain devoid of xylose and fucose (71).

Therefore, neither Art v 1 nor Art v 2 seem able to widely cross-react with classical glycan chains such as

those found in grass or olive pollens. Recently, a cross-reactivity has been observed between Art v 1 and the timothy grass pollen allergen Phl p 1: this could be related to the defensin-like domains shared by these proteins rather than to their respective glycans (72).

Mugwort pollen was nevertheless shown to take part in glycan-related cross-reactions (59) and its HMW proteins (40-80 kDa) are highly suspected to be glycosylated (42, 61, 64, 73). But mugwort pollinosis being most often associated with other pollen sensitizations, the quantitative effect of mugwort in promoting the synthesis of anti-CCD IgE is not clear.

Lamb's quarter, plantain, and pellitory pollens contain glycosylated major allergens (Che a 1, Pla l 1, and Par j 1, Par j 2, and Par o 1, respectively), and a polygalacturonase is found in oilseed pollen. The glycan-related IgE-reactivity of these allergens seems weak (22, 74-77).

■ **Plant foods:** CCD-related cross-reactions between pollens and plant foods, or among plant foods, are frequently observed. Van Ree pointed that anti-CCD IgE are rarely present in plant food allergic patients without an associated pollinosis (4).

For example, among 11 patients non exposed to Nangai nut but positive in vitro to that nut, 9 were allergic to birch, grass and/or mugwort (78): in these patients bromelain was able to inhibit the IgE-reactivity to Nangai nut.

➤ **In which plant foods CCD have been characterized?**

Several protein families are glycosylated in plant foods (see fig. 3). Legume seeds, for example, contain vicilins 7S globulins (peanut Ara h 1, lentil Len c 1.01, etc.), peroxidases and cysteine proteases: CCD-driven reactions are to be expected with these products.

CCD were demonstrated in tomato (34, 79), in celery (59, 80), in zucchini and other cucurbitaceae plants [81, 82]. Tomato peroxidase and Lyc e 2, cherry Pru av 2, hazelnut Cor a 11, cereal amylase/trypsin inhibitors, garlic alliin lyase, and soybean Gly m Bd 30K contain cross-reactive glycans (83-89). Cereal germins and germin-like proteins are periodate-sensitive (90). Cross reactions between pollens and 30-80 kDa food proteins strongly suggest the presence of CCD in apple (42, 73), kiwi (42, 62, 73), cherry (65), mango (60), celery (66), carrot (64), poppy seed (61) and sharon fruit (63).

One should notice that heat does not abolish glycan-related IgE-reactivity (91) and that potato patatin, as well as many other tuber storage proteins, are glycosylated (92).

➤ **How frequent are CCD-related reactions in the case of plant foods?**

Few data are available:

- Anti-CCD IgE were demonstratable in 10 to 50% patients sensitized to tomato, carrot, celery, or zucchini (5, 93);

- Ebo *et al.* studied 82 pollen allergic patients who had a positive CAP result for bromelain (37): a positive CAP result for apple was 3.5 more frequent in patients non-allergic to apple than in true allergic ones;
- Van der Veen *et al.* found 29 CCD-positive sera in 32 patients who were allergic to grass pollens and RAST positive (but skin test negative) to peanuts (94).
- More studies are thus needed to better define how frequent are CCD-related reactions in the case of plant foods.

■ **Latex:** CCD-based reactions between hevea latex and other plant-derived products, such as pollens, were to be expected. Indeed, the prevalence of latex sensitization in unselected populations is higher with *in vitro* tests than with skin testing (95): Merrett *et al.* found a RAST-positive rate as high as 7.3% in blood donors (96). In addition, this rate was clearly influenced by the exposure to pollens (7.3% during summer vs 3.9% during winter) (96). A significant correlation was also noted between latex and ryegrass quantitative results *in vitro* ($r=0.7$) (97).

A CCD interference in pollen allergic patients is strongly suggested by some data :

- ryegrass, mugwort, and ragweed pollens were able to inhibit latex in patients with an unexpected sensitization to latex but not in already known latex allergic patients (98);
- a bromelain RAST was positive in 17 out of 21 patients RAST-positive but not allergic to latex; conversely, none of latex-allergic but non pollinic patients had a positive bromelain RAST result (37).

To explain this CCD interference some glycoproteins should be present in latex. Among latex proteins, Hev b 2 has been the only known glycosylated allergen for a long time. The IgE-reactivity of this beta 1,3-glucanase was demonstrated to be periodate-sensitive (99). Recently, a novel glycoprotein, named Hev b 13, was characterized in latex and found as a major allergen (100). Hev b 13 could have a significant impact on *in vitro* results because its reactivity seems restricted to its glycan chains (101).

■ **Non-plant products:** Apart from hymenoptera venoms, scarce data are available for non-plant products. This is partly due to the lack of a glycoprotein model as adequate as bromelain is for plant-derived products. Therefore, negative results observed during inhibition assays of a non-plant product by bromelain cannot definitely prove the absence of glycan-related reactivity in the tested product.

➤ **Mammals:** Lipocalins are potent allergens in hair, fur, dander and/or urine of several animals (102). Some of them are glycosylated, such as dog Can f 1, mouse Mus m 2, horse Equ c 1, or rabbit Ory c 1 (103). But mouse Mus m 1, guinea pig Cav p 1 and Cav p 2,

and cow Bos d 2 are not glycosylated (103, 104). Data are lacking about newly characterized cat lipocalin Fel d 4 (105). When studied (106), the glycan-related IgE-reactivity of glycosylated lipocalins has been found low, but more studies are needed.

Non-lipocalin allergens, such as cat Fel d 1 or horse Equ c 4 are also glycosylated. Fel d 1 IgE-reactivity was found periodate-sensitive, but this result has been questioned on the grounds of a possible effect on the secondary structure of the protein (107).

In cow milk, glycan chains have been demonstrated on alpha-lactalbumin, lactoferrin, and immunoglobulins. Some caseins show an O-glycosylation (108). These allergens seem devoid of glycan-related IgE-reactivity.

➤ **Birds:** In white of hen's egg two main allergens are glycosylated: ovomucoid Gal d 1 and ovalbumin Gal d 2 (109). The eggs of duck, quail, etc. contain similar allergens. Ovomucoid glycans were found unreactive with IgE (110). Egg yolk main allergens, and their counterpart in feather dust, are albumin-like livetins: these proteins are not glycosylated (111).

➤ **Fish:** Fish parvalbumins contain an atypical glycosylation (112), unable to cross-react with plant glycans (70).

➤ **Crustacea:** Crustacea main allergens are tropomyosins and arginine kinases. These protein families are also found in other invertebrates, such as mites and insects (113, 114). Conflicting results were obtained regarding the glycosylation of tropomyosins (115, 116). An N-glycosylation site was noted in arginine kinases. But any glycan-induced IgE reactivity of these proteins has not been observed up to now.

➤ **Mollusks:** Some glycoproteins in mollusks might cross-react with plant CCD (70, 71): For example, *Helix pomatia* hemocyanin is xylosylated (117) and squid rhodopsin is 1,3-fucosylated (118). A cross-reactivity between mussel and some plant foods was reported in a patient (119).

➤ **Mites:** Mites are important sources of allergens. Among mite families of allergens, some are glycosylated: group 7 (Der p 7 and Der f 7 [120, 121]), group 11 (Der f 11) (122, 123), group 13 (Aca s 13) (124), and group 18 (Der f 18) (125). But this glycation is variable, depending on mite species: Lep d 7 (126), Lep d 13 (126) and Blo t 13 (127) are not glycosylated.

The case of mite group 1 allergens has been especially studied. These major allergens (Der p 1, Der f 1,...) belong to the same protein family as bromelain, the papain-like family of cysteine proteases (128). Although glycosylated (129, 130), their glycan-related IgE reactivity is probably negligible (71), as shown by results obtained with recombinant Der p 1 or Der f 1 (129, 131-133). In addition, most patients sensitized to mites but not to pollens had negative bromelain RAST results (1, 134).

➤ **Hymenoptera venoms:** Some hymenoptera venom allergens are glycosylated (hyaluronidases, phospholipases A1 and A2) (135). Whereas hyaluronidases are found in Apidae and Vespidae (Api m 2, Ves v 2, etc.), phospholipases A2 (PLA2) are only present in honeybee (Api m 1) and bumblebee venoms. These allergens have no xylose but their 1,3-fucosylation makes them susceptible to some cross-reactivity with plant CCD (39, 136).

Such a possibility is suggested by several findings.

- high prevalence rates of positive RAST results were found in unselected populations: 24.8% for honeybee and 8% for wasp (137). And among honeybee positive patients, 28% were also bromelain positive (136);
- an *in vitro* double positivity for honeybee and for wasp was observed in 30-50% cases (138, 139), a rate obviously higher than doubly stung patients;
- bromelain was able to inhibit honeybee and wasp RASTs (138) and also PLA2 (136);
- periodate could inhibit honeybee bands on immunoblots of patients stung only by a wasp (139).

Because they are present in both Apidae and Vespidae, hyaluronidases are suspected to cause most of these glycan-induced cross-reactivities (135, 139, 140). For example, deglycosylation affected IgE-reactivity of honeybee hyaluronidase Api m 2 but not that of PLA2 (135).

What is the origin of the anti-glycan IgE observed in patients positive for hymenoptera venoms? This important point is still debated:

- some authors think that the sensitization to hymenoptera venoms is able by itself to induce a CCD-reactivity. For example, Mairesse *et al.* noted a positive bromelain RAST only in patients with double positive hymenoptera skin tests (141);
- others authors think that the CCD-reactivity is mainly driven by a concurrent pollen sensitization [3, 37] especially a sensitization to grass pollens (137).

Data are lacking regarding glyco-epitopes in ant venoms, but one should notice that they contain phospholipases A1 allergens like wasp venoms (142).

➤ **Insects:** The presence of xylose-bearing glycoproteins was demonstrated with a specific IgG antibody in several insect extracts: American cockroach, *Triatoma*, flies, etc. (13, 70, 143). The IgE-reactivity of a silverfish extract was found periodate-sensitive (144). A 1,3-fucosylation was sometimes found and could explain some cross-reactivity with plant CCD (71). One could expect a glycan-related IgE-reactivity in mosquito saliva which contains hyaluronidases. This has not been studied so far.

➤ **Helminths:** Few data are available about glycan-related IgE-reactivity of helminths (145). Some helminth proteins bear glycan chains with 1,3-fucose and/or 1,2-xylose (11, 146-150). An IgE reactivity, possibly O-glycan-related, was observed with *Anisakis* (151, 152). Cross-reactivity with plant CCD has not been studied.

➤ **Fungi:** Moulds and yeasts are rich in glycoproteins which are mainly mannosylated and devoid of xylose [14]. Most fungi exhibit a glycan-related IgE-reactivity (14). Major allergens such as baker's yeast alpha amylase Asp o 21, *Aspergillus* Asp f 2, *Cladosporium* Cla h 2, *Alternaria* Alt a 1 or *Penicillium* Pen ch 20 are glycosylated (153). *Epicoccum* Epi p 1 and fungal alkaline serine proteases (*Penicillium* and *Aspergillus* group 13 allergens, *Curvularia* Cur l 1) showed periodate-sensitive IgE-binding (154-157).

Mannoproteins were shown responsible for cross-reactions between numerous fungi, especially with *Candida albicans* and *Malassezia sympodialis* (158, 159). However, these fungal CCD did not cross-react with plant glycans (70).

The references will be published in the next issue of the European Annals of Allergy and Clinical Immunology, together with the second part of this article. Data for and against any clinical impact of carbohydrate epitopes, and practical issues in allergy diagnosis will be discussed in this second part.

