

THE INFLUENCE OF CARBOHYDRATE STRUCTURES PRESENT IN COMMON ALLERGEN SOURCES ON SPECIFIC IGE RESULTS

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Background: Cross-reactive carbohydrate determinants (CCD) are well known interferants in specific IgE assays (sIgE). Glyco-epitopes are not restricted to CCD and extracts used to prepare *in vitro* tests contain many other glycoproteins able to bind glycan-specific IgE. The overall amounts of IgE-bindable glycan structures in allergen sources are unknown.

Objective: We aimed at quantifying the influence of N-glycan structures on IgE reactivity to commonly tested allergen sources.

Methods: IgE reactivity to 51 allergen extracts, one purified natural allergen and 10 recombinant allergens was measured on Phadia UniCAP system using 2 sera demonstrating significant levels of glycan-related IgE reactivity. Immobilized bromelain and horseradish peroxidase (HRP) were used to capture N-glycan-specific IgE from these sera. Residual IgE reactivity was measured for 42 allergen sources and 4 recombinant/purified allergens.

Results: An obviously excessive number of positive CAP-results were obtained with both sera, especially for plant-based allergen sources. Capture of glycan-specific IgE led to a decrease of serum IgE reactivity, variable among allergen sources and between sera. Among others, peanut results were proven largely interfered by the presence of glycan-specific IgE. Unexpectedly some allergen sources showed a slight influence of glycan-related reactivity, such as cockroach, mosquito, mussel, shrimp and domestic mites.

Conclusion: In patients sensitized to pollens or to Hymenoptera venoms sIgE results should be interpreted with caution. One cannot subtract the result of a glyco-reporter test (bromelain and/or HRP) in order to compute glycan-free sIgE results for common allergen sources like peanuts. As long as the demonstration of a significant role for glycan structures in clinical allergic reactions is lacking, a simple pre-treatment able to discard glycan-specific IgE from serum would be useful to improve accuracy of *in vitro* diagnostic tests.

Key-words: Allergy diagnosis - Specific IgE - CCD - Glycoproteins - Peanuts.

INTRODUCTION

Many proteins bear carbohydrate structures. Glycoproteins, whether allergenic or not, are thus regular components of extracts used for skin testing or for *in vitro* specific IgE assays (sIgE).

Glycan structures can give rise to IgE antibodies in susceptible subjects. These IgE antibodies are able to recognize similar glyco-epitopes on different glycoproteins. Cross-reacting glyco-epitopes are often called "cross-reactive carbohydrate determinants" (CCD) (1).

Why glycan structures and CCD are worth studying in the field of allergy diagnosis? (2).

➤ Cross-reactivity between CCD is potentially wider than between peptide epitopes because CCD ignore protein families boundaries [3].

➤ sIgE *in vitro* tests are prone to cross-reactivity because IgE antibodies are free to move in serum and can bind to any compatible structure in the assay extract. In contrast, cellular tests, such as skin tests,

are less prone to cross-reactivity because cellular activation needs the presence of two adjacent and compatible pairs of epitopes and IgE (4). Considering that bi- or multi-valency is rare among glycoproteins regarding glyco-epitopes, most experts agree on a poor, if any, clinical relevance of glycan-specific IgE (5).

➤ The presence of glycan-specific IgE in serum is not a rare event : based on a positive result for a glyco-reporter such as bromelain, as many as 15-40% pollinergic patients and Hymenoptera venom allergic subjects do possess such IgE antibodies (6, 7).

Thus sIgE assays are at risk to be interfered by the presence of glycan structures in the extract. Some molecules known to sensitize exposed subjects only in very rare and specific conditions (eg. bromelain) or regarded as non allergenic (eg. horseradish peroxidase or ascorbate oxidase) are currently used as glyco-reporters in daily practice : a positive *in vitro* result for such a molecule indicates the presence of glycan-specific IgE in the patient's serum (8).

This information is useful but raises many questions not well studied up to now : do different glyco-reporters give equivalent results in a given patient? Are all aller-

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gen sources equally susceptible to the presence of glycan-specific IgE in the patient's serum? Can the quantitative result for the glyco-reporter be subtracted from the result of other allergen sources (eg. peanuts)? We aimed at exploring the extent of N-glycan-related IgE reactivity towards a large panel of common allergen sources.

MATERIAL AND METHODS

■ **Sera:** Among a panel of 36 sera (Scipac Ltd, UK) included in a multicentric analytical study of sIgE assays (9) we selected two sera showing a significant reactivity to molecules commonly used as glyco-reporters, ie. bromelain and horseradish peroxidase (HRP).

■ **sIgE determinations:** IgE-reactivity of these two sera was measured on Phadia UniCAP 100 system using 51 allergen sources (24 foods, 16 pollens, 4 insects, 2 moulds, 2 mites, 2 pets, and latex), 10 recombinant allergens, and 1 purified natural allergen.

The extent of CCD-reactivity for most of these allergen sources and recombinant/purified allergens was studied. For that purpose we designed an immuno-capture assay : 150 µl/well of biotinylated bromelain and horseradish peroxidase (HRP) Immulite reagents (DPC, Los Angeles) in a 4 :1 ratio were used to coat streptavidin-based plates (Nunc 436014). This ratio was chosen to compensate for the number of glycan chains present on bromelain and HRP (1 and 4, respectively). After a wash and dry step, glycan-specific IgE were captured from sera (100 µl/well) for 8 hours at room temperature. Sera were diluted aiming at a glycan-related reactivity before capture of 1,5 kU/l. Allergen sources giving a result < 1 kU/l before capture were not tested. IgE reactivities before and after capture were quantified during the same assay run.

RESULTS

■ **Patients' sera:** Serum n°1 and serum n°2 differ on many aspects : serum n°1 has a low level of total IgE (30 kU/l) and a higher reactivity to HRP (13,9 kU/l) than to bromelain (12,1 kU/l). In contrast, serum n°2 has a high level of total IgE (1330 kU/l) and a clearly lower reactivity to HRP compared to bromelain (7,8 kU/l vs 28,5 kU/l).

➤ **CAP-Results obtained for allergen sources:** As shown in Table 1, CAP results are strikingly similar among plant sources for serum n°1 but vary from one source to another one for serum n°2. Both sera demonstrated an obviously excessive of positive results, a characteristic for a CCD interference.

➤ **CAP-Results obtained for recombinant/purified allergens:** Glycan-driven reactivity is avoided with

pure unglycosylated allergens. This is the case for the 11 recombinant molecules tested in the present study (Table 2). Low results for serum n°1 indicates that most of IgE reactivity observed with pollens, plant foods, latex and Hymenoptera venoms comes from a CCD interference. This is in line with the clearly positive result obtained with nOle e 1, a purified naturally glycosylated allergen. Serum n° 2 adds two kinds of IgE reactivity: a sensitization to pollens and *Alternaria* and a CCD reactivity most probably induced by the sensitization to pollens. Part of the IgE reactivity to pollens other than grass could come from a sensitization to profilins and polcalcins. Results for rBet v 2 and rPhl p 12 and for rBet v 4 and rPhl p 7 are very close to each other.

➤ **Results of the immuno-capture assay:** Residual reactivity of IgE anti-bromelain and IgE anti-HRP demonstrated that glycan-specific IgE reactivity capture was nearly total with serum n°1 but was subtotal with serum n°2 : around 10% glycan-specific IgE reactivity remained uncaptured in serum n°2 with our assay conditions (Table 3).

Capturing glycan-specific IgE from serum n°2 had a variable effect on the result of common allergen sources. This effect can be divided into 3 categories (Figure 1):

- greatly influenced by the presence of IgE-reactive glycan structures : pollens, plant foods, latex, hymenoptera venoms;
- unaffected : mammal-derived products, fish, egg, molds and unglycosylated recombinant allergens;
- partly interfered : cockroach, mosquito, mites, mussel, and shrimp. A single but glycosylated molecule like olive allergen nOle e 1 is also partly reactive through its glycan chains.

Capturing glycan-specific IgE from serum n°1 led to virtually no reactivity for all testable allergen sources (Table 3).

DISCUSSION

Our results demonstrate a significant glycan-related interference regarding plant sources, latex, and hymenoptera venoms. Extracts from mammals, fish, egg, and molds and unglycosylated recombinant allergens are free from this interference.

Purified natural allergens are at risk of CCD interference if they bear glycan structures. Great care should be taken when interpreting results of such single molecules: nOle e 1, as demonstrated in our study, but also other UniCAP tests such as to nPhl p 4 (timothy grass pollen) or nApi m 1 (honeybee) (10).

Some allergen sources seem partly influenced by the presence of anti-glycan IgE: cockroach, mosquito, mussel, shrimp, and domestic mites. Although data

	Allergen source	Code	Serum n°1	Serum n°2
Mites	D. pteronyssinus	D 1	0.57	1.39
	D. farinae	D 2	2.32	2.09
Pets	Cat	E 1	0.21	6.15
	Dog	E 5	1.3	4.11
Moulds	Aspergillus	M 3	0.47	14
	Alternaria	M 6	0.4	48.5
Grass pollens	Bermuda grass	G 2	14.2	79.2
	Orchard grass	G 3	14.1	176.2
	Ryegrass	G 5	14.2	193.4
	Timothy grass	G 6	14.6	180.4
	Wheat pollen	G 15	13.2	116.8
Tree pollens	Birch	T 3	11.9	24.8
	Hazel	T 4	11.5	28.9
	Olive	T 9	13.7	41.9
	Plane tree	T 11	13.9	35.2
	Ash tree	T 15	14.3	29.9
	Cypress	T 23	12.5	9.15
Weed pollens	Ragweed	W 1	13.1	40.6
	Mugwort	W 6	12.8	25.6
	Plantain	W 9	13.2	27.1
	Lamb's quarter	W 10	12.7	22
	Pellitory	W 21	14	11.6
Hymenoptera venoms	Honeybee	I 1	14.2	7.83
	Yellow jacket	I 3	11.5	3.71
Insects	German cockroach	I 6	11.2	6.23
	Mosquito	I 71	1.95	2.86
Latex	Hevea latex	K 82	15	7.74
Foods of animal origin	White of egg	F 1	0.26	0.37
	Egg yolk	F 75	0.16	0.27
	Cow's milk	F 2	0.22	0.31
	Beef meat	F 27	0.21	0.39
	Cod	F 3	0.28	0.52
	Shrimp	F 24	2.19	1.52
	Mussel	F 37	1.16	2.63
Plant foods	Wheat flour	F 4	12.2	26.4
	Sesame	F 10	14.5	23.6
	Peanuts	F 13	14.9	26.5
	Soyabean	F 14	12.6	11.3
	Hazelnut	F 17	14	7.13
	Almond	F 20	12.5	5.8
	Tomato	F 25	14.3	27
	Carrot	F 31	13.6	23.7
	Potato	F 35	13.8	25.3
	Apple	F 49	11.9	8.13
	Kiwi	F 84	14.1	26.9
	Celery	F 85	14.4	29
	Mustard	F 89	10	4.67
	Banana	F 92	11.1	19
	Peach	F 95	11.8	13.7
	Avocado	F 96	14	14
	Walnut	F256	10.5	6.14

Table 1: Results (kU/l) obtained for 51 allergen sources with 2 CCD-positive sera

Allergen source	Allergen *	Code	Serum n°1	Serum n°2
Birch pollen	rBet v 1	T215	0.57	0.81
	rBet v 2	T216	0.59	3.85
	rBet v 4	T220	0.67	15.6
Latex	rHev b 1	K215	0.42	0.65
	rHev b 3	K217	0.47	0.9
	rHev b 5	K218	0.25X	0.45
	rHev b 6.02	K220	0.4	0.91
Olive pollen	nOle e 1	T224	14.8	18.8
Timothy grass	rPhl p 7	G210	0.43	13.7
	rPhl p 12	G212	0.69	4.47
	rPhl p1+5b	G213	0.59	155.2

Table 2: Results (kU/l) obtained for 11 individual allergens with 2 CCD-positive sera.

* r = recombinant / n = natural (purified).

		Bromelain	HRP *
Serum n°1	Before capture	12.1	13.9
	After capture	0.39	0.38
Serum n°2	Before capture	28.5	7.8
	After capture	2.99	0.69

Table 3: Results for glyco-reporter molecules on UniCAP (kU/l) before and after immunocapture of glycan-related IgE reactivity.

*Horseradish peroxidase.

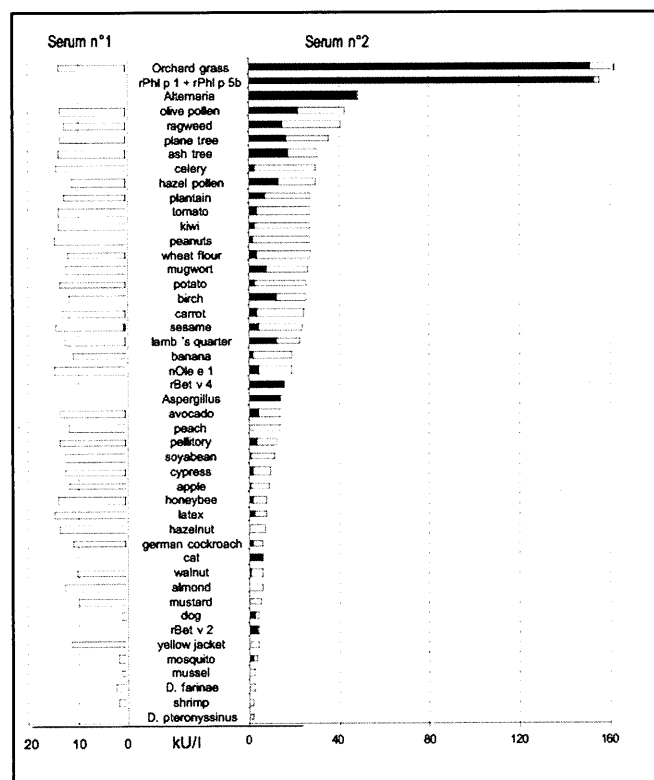


Figure 1: Effect of immuno-capture of glycan-specific IgE on 42 allergen sources and 4 individual allergens.

Light grey bars indicate the result obtained before immuno-capture. Dark grey bars represent the IgE reactivity left after immuno-capture

about glycomes of invertebrates are still scarce (11, 12), one can suspect the presence of N-glycan structures bearing a core fucose-1,3 in these allergen sources. Recent works confirmed that shrimp and mites extracts contain IgE-reactive carbohydrate components (13, 14). These findings question the classical view which states that animal allergen sources, except Hymenoptera venoms, do not bear carbohydrates able to cross-react with plant glycan structures. For instance, no *in vitro* reactivity to bromelain was found in (non pollinic) patients allergic to domestic mites [6, 15]. But the reverse was not studied, ie. how many positive results for mites are present among CCD-positive subjects. Presumably plant-driven glycan-specific IgE have a weak affinity for mites carbohydrate structures. As a result, only high titers of anti-CCD IgE, such as those of the sera we tested in the present work, produce a substantial effect on *in vitro* results for these allergen sources.

In order to improve the measurement of clinically relevant IgE, the application of (unglycosylated) recombinant allergens has the priority over classical extracts as long as the loss of sensitivity is negligible or largely compensated by a better specificity : for example testing rBet v 1 instead of birch, rPhl p 1 and rPhl p 5b instead of timothy grass, or rHev b 1 + rHev b 5 and/or rHev b 6.02 instead of hevea latex.

The Bromelain/HRP reactivity ratio is not constant from one patient to another one : here 0.9 for serum n°1 and 3.6 for serum n°2. Preliminary tests (data not shown) demonstrated that HRP only could capture all IgE reactivity to bromelain in serum n°1 and that bromelain was the main driver of CCD reactivity in serum n°2.

Bromelain and other glyco-reporter molecules such as HRP cross-react with each other more or less, depending on patient's IgE affinities towards xylose or core 1,3-fucose on glycan chains (5).

Each patient has his/her own spectrum of glycan-related IgE reactivity. One cannot simplify glycan-related IgE reactivity to bromelain only.

As shown by the variable effect of capturing glycan-specific IgE on different allergen extracts, one cannot subtract bromelain and/or HRP reactivity (kU/l) from results obtained with other sources in order to compute a glycan-corrected ("true") IgE reactivity.

Results of the present study thus add to the already known drawbacks of the published cut-offs for allergy diagnosis, such as 15 kU/l for peanuts (16-18).

Although an infrequent finding, some sera show an IgE reactivity restricted to glycan structures only (19). This is the case of serum n°1 in the present study. When assayed with devices testing multiple allergen sources (eg. MAST® Allergy Test), such sera give an unexpected high number of positive results. But, if wisely interpreted, this finding becomes a useful piece of information, a warning about the occurrence

of anti-CCD IgE in the patient's serum. Such a piece of information is missed when testing only a couple of allergen sources as it is usual to do with discrete analyzers like the UniCAP system.

The interference of carbohydrate structures on *in vitro* tests is a general phenomenon, obviously not specific to Phadia UniCAP reagents. Preliminary data obtained in our laboratory with the same sera as those used in the present study showed some degree of CCD reactivity with other analytical platforms like Siemens Immulite 2000 or ADVIA Centaur.

As long as the demonstration of a significant role for glycan structures in clinical allergic reactions is lacking, sIgE *in vitro* assays should avoid measuring any glycan-related IgE reactivity. Before a large array of unglycosylated recombinant and/or purified natural allergens is available for routine use, *in vitro* results for CCD-prone allergen sources should be interpreted with caution. Testing glyco-reporters, such as bromelain and HRP, can help. But capturing glycan-specific IgE before measuring serum IgE reactivity is clearly the best option (5). There is a strong need for a reliable assay that exclusively measures clinically relevant IgE. An easy and simple way to deplete serum for glycan-specific IgE is currently under investigation in our laboratory.

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Press Releases

CHRONIC COUGH CAN BE TAMED WITH THE RIGHT TREATMENT

ARLINGTON HEIGHTS, IL — Chronic cough - often causing much discomfort, frustration, irritability and sleep disturbances in 10 percent to 20 percent of adults - is one of the leading reasons for consultation with a physician according to a report published this month in *Annals of Allergy, Asthma & Immunology*, the scientific journal of the American College of Allergy, Asthma and Immunology (ACAAI).

Matthew A. Rank, M.D., at Mayo Clinic in Rochester, Minn., and his colleagues, define a chronic cough as one lasting longer than 8 weeks in adults and 4 weeks in children.

"Coughing plays an important role in the body's defense system by clearing the airway of excess secretions and foreign bodies", note the authors. "However, in some patients cough may be excessive and unnecessary and may lead to a general deterioration in quality of life". From the patient's perspective, the most prevalent problems observed in a recent survey were frustration, irritability, anger, frequent physician visits and testing, and sleep disturbances.

The three leading causes of chronic cough in adults are upper airway cough syndrome (UACS, also known as postnasal drip syndrome), asthma, and gastroesophageal reflux disease (GERD). The treatment described as the most effective cough resolution involves testing for all three conditions and treating them simultaneously.

"Unfortunately, less research is available in childhood cough when compared to their adult counterparts", said co-author John Oppenheimer, M.D., UMDNJ-New Jersey Medical School, Cendrar Knolls, N.J. "In one study bacterial bronchitis was identified as the most common explanation for chronic cough in children, while asthma, GERD and UACS comprised less than 10 percent of the diagnosis. On the other hand, in another study, asthma was found to be the most common cause of chronic cough in children, reinforcing the need for further research in identifying causes of cough in this population".

New diagnostic techniques and treatments in development may bring faster relief to cough sufferers. Measurement of exhaled nitric oxide is shown to correlate with airway inflammation for identifying asthma. A portable Aeriflux testing system has been developed that may allow easier diagnosis of GERD-associated cough.

Taming the chronic cough requires a thorough evaluation. Considering the leading causes of chronic cough, authors note that allergists should be pivotal in its evaluation and management.

Patient information on allergic diseases including asthma is available by calling the ACAAI toll free number at (800) 842-7777 or visiting its Web site at www.acaai.org <<http://www.acaai.org>>. The American College of Allergy, Asthma and Immunology (ACAAI) is a professional medical organization headquartered in Arlington Heights, Ill., that promotes excellence in the practice of the subspecialty of allergy and immunology. The College, comprising more than 5,000 allergists-immunologists and related health care professionals, fosters a culture of collaboration and congeniality in which its members work together and with others toward the common goals of patient care, education, advocacy and research.