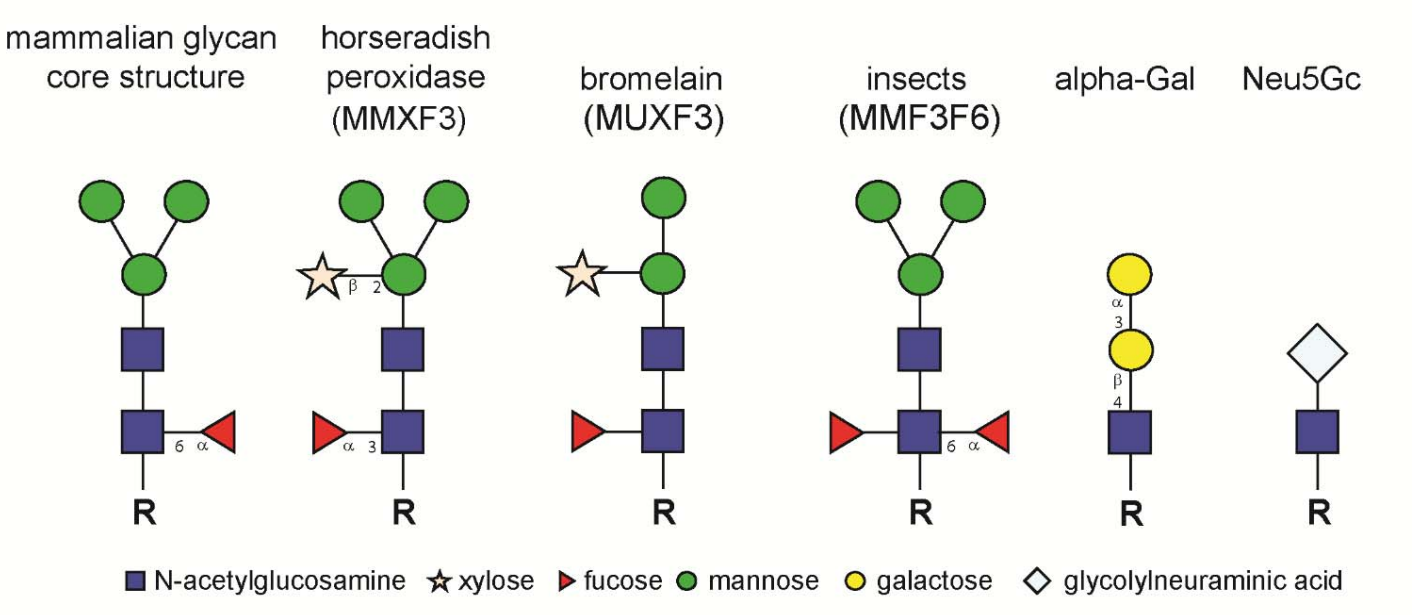
Cross-reactive carbohydrate determinants and allergenicity

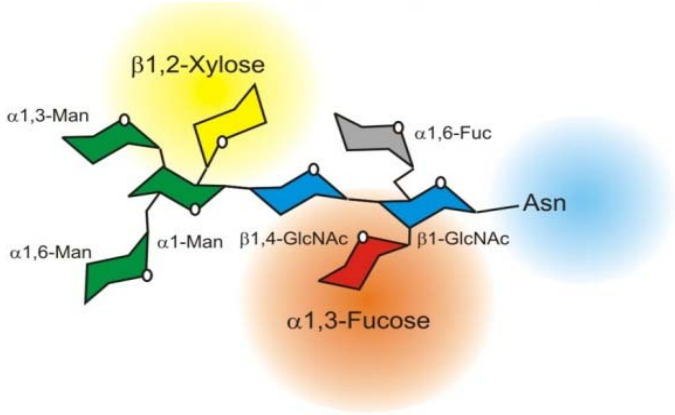
Edzard Spillner, Aarhus

What are cross-reactive carbohydrate determinants?

- Insects and plants, but also mammals generate variant carbohydrate patterns
- Highly abundant, xenobiotic core modifications form dominant epitopes
- Strong IgE and IgE responses

- Several CCDs have been reported

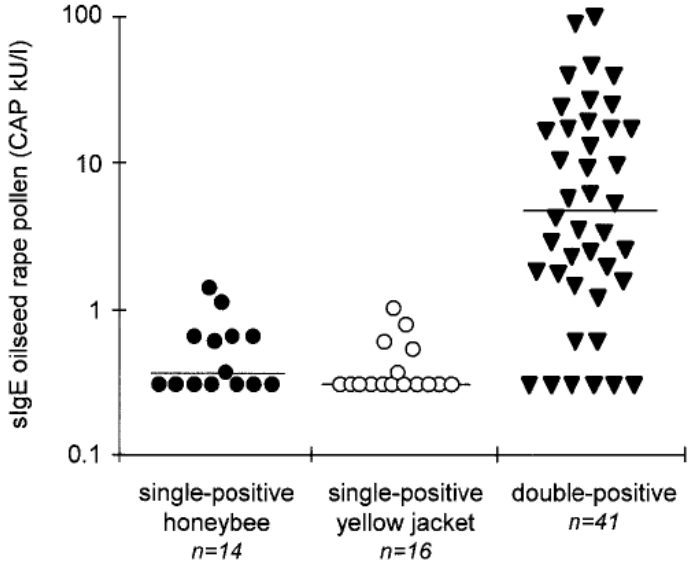


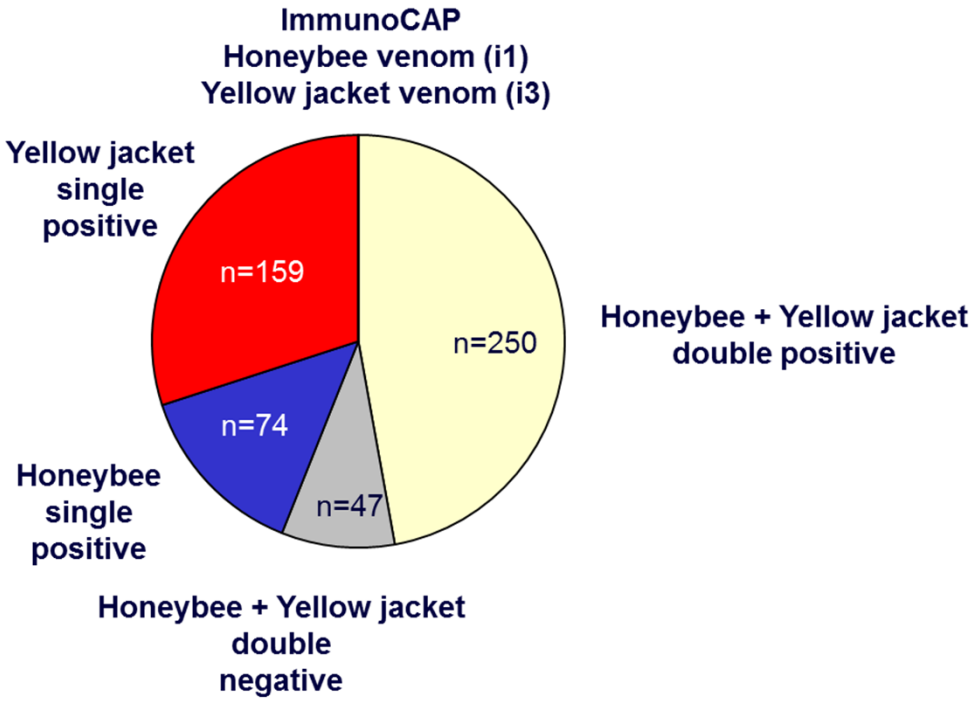


Is IgE reactivity protein-directed only?

- sIgE reactivity often is mediated to CCDs
- Correlation of sIgE with clinical picture limited

- Information about glycosylation important

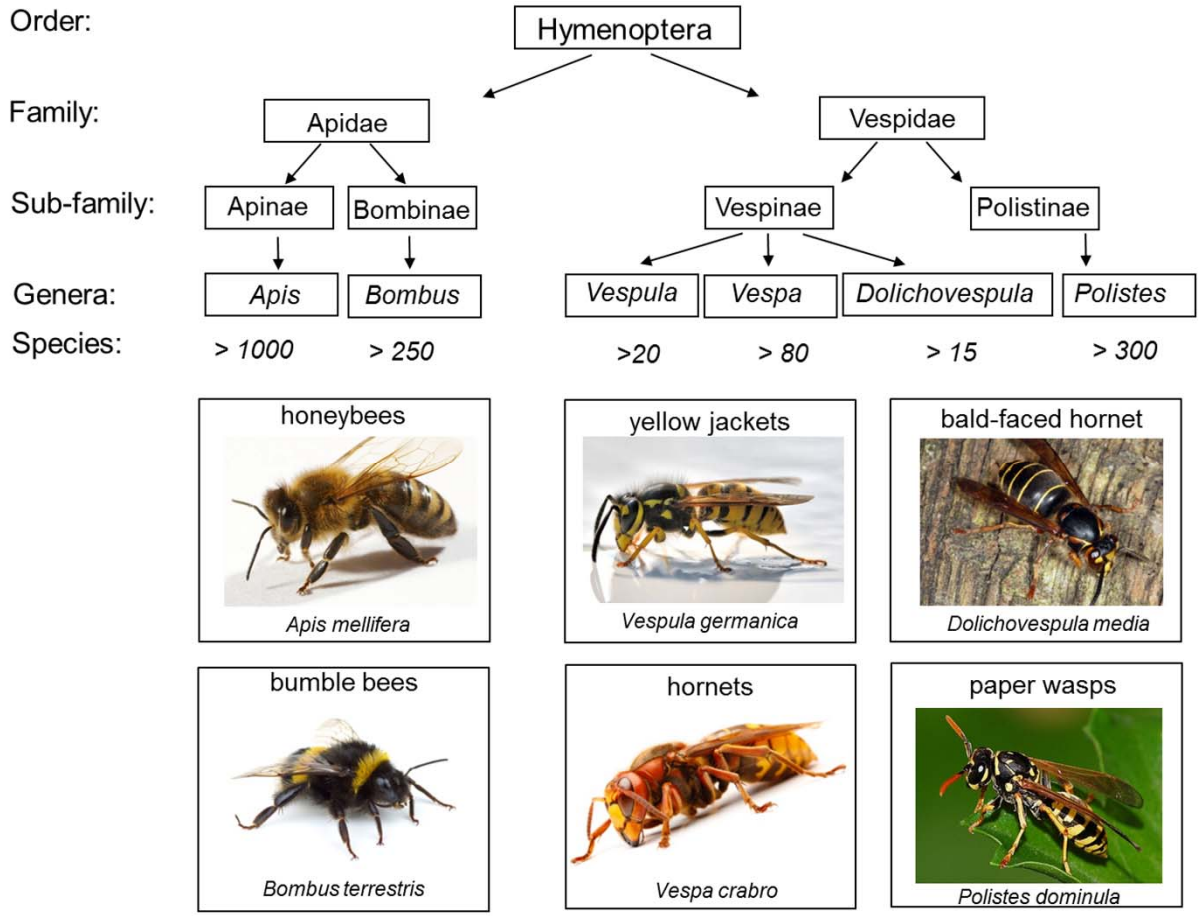




Hemmer et al., J. Allergy Clin. Immunol., 2001; Jakob et al.,Molecular Allergy Diagnostics, 2017

Hymenoptera venom: large diversity of species

- Main culprits blong to different families
- Honeybee and yellow jacket



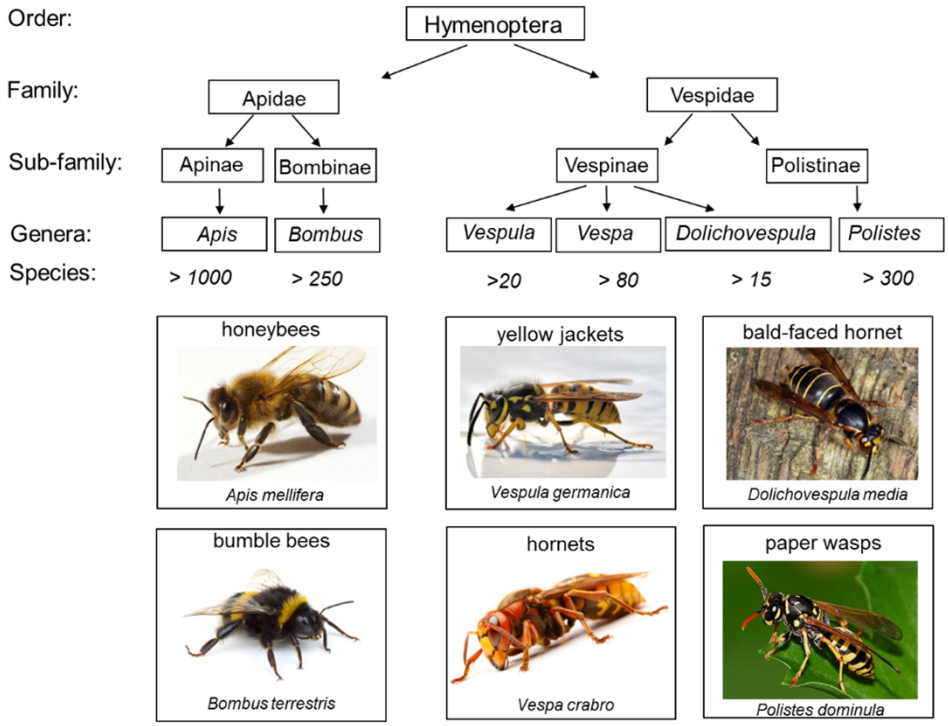
Jakob et al., Curr Opin Allergy Clin Immunol, 2018

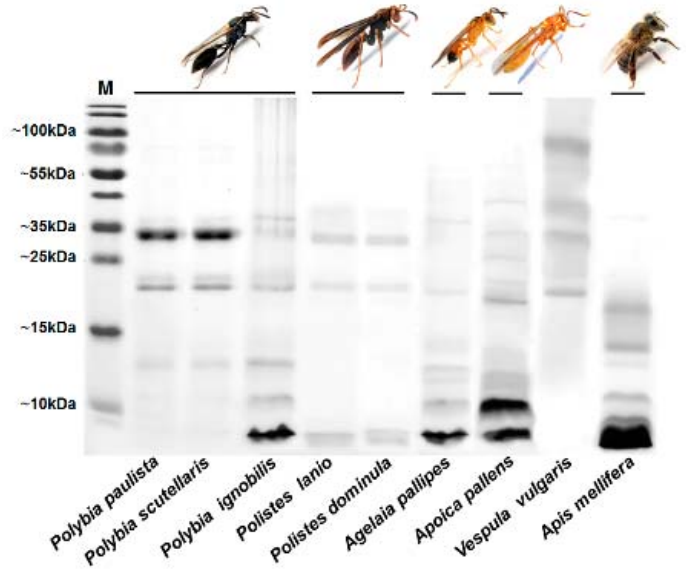
Beyond extracts: HBV and YJV allergens today

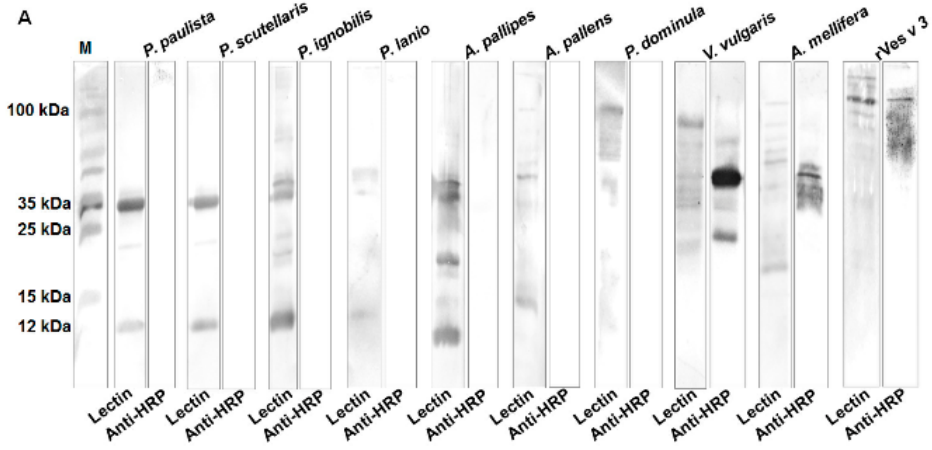
Allergen	Name/function	MW (kDa)	% DW	Potential N-glycosylation	Eukaryotic expression
BEES (*Apis mellifera, A. cerana, A. dorsata*)					
Api m 1, Api c 1, Api d 1	Phospholipase A2	17	12	1	+
Api m 2	Hyaluronidase	45	2	3	+
Api m 3	Acid phosphatase	49	1–2	2	+
Api m 4	Melittin	3	50	0	–
Api m 5	Allergen C/DPP IV	100	<1	6	+
Api m 6	Protease inhibitor	8	1–2	0	+
Api m 7	Protease	39	?	3	+
Api m 8	Carboxylesterase	70	?	4	+
Api m 9	Carboxypeptidase	60	?	4	+
Api m 10	CRP/icarapin	55	<1	2	+
Api m 11.0101	MRJP 8	65	?	6	+
Api m 11.0201	MRJP 9	60	?	3	+
Api m 12	Vitellogenin	200	?	1	+
YELLOW JACKETS (*Vespula vulgaris, V. flavopilosa, V. germanica, V. maculifrons, V. pensylvanica, V. squamosa, V. vidua*)					
Ves v 1, Ves m 1, Ves s 1	Phospholipase A1	35	6–14	0, 0, 2	+
Ves v 2.0101, Ves m 2	Hyaluronidase	45	1–3	4	+
Ves v 2.0201	Hyaluronidase[a]	45	?	2	+
Ves v 3	DPP IV	100	?	6	+
Ves v 5, Ves f 5, Ves g 5, Ves m 5, Ves p 5, Ves s 5, Ves vi 5	Antigen 5	25	5–10	0	+
Ves v 6	Vitellogenin	200	?	4	+

CCD variations within one family

- Polistinae allergens are devoid of CCDs



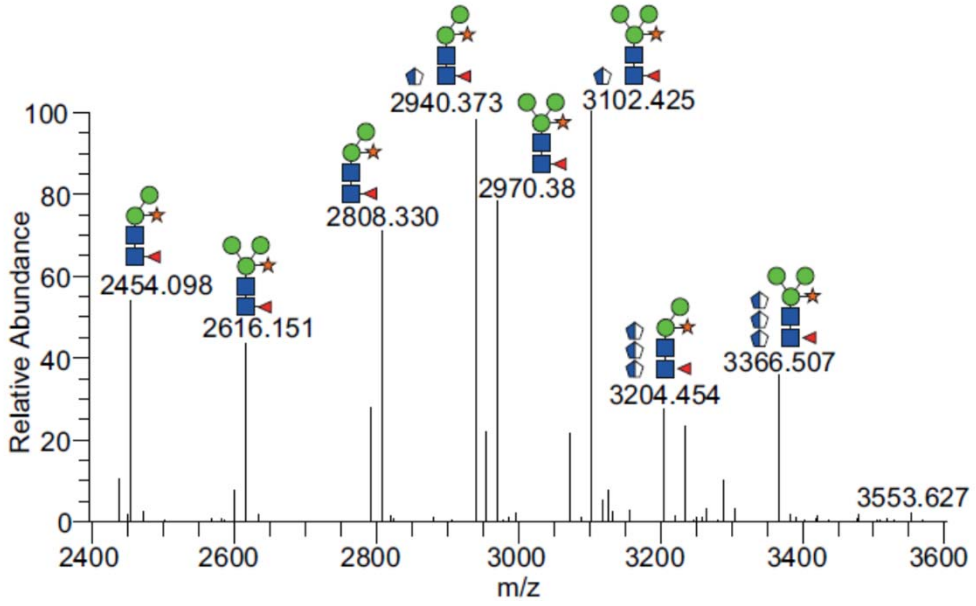




Perez-Riverol et al., Mol Immunol 2017, Perez-Riverol J Allergy Clin Immunol 2017

CCD heterogeneity on pollen, food, biologicals

- E.g. Phl p 1 (core modification) and cetuximab (alpha-Gal)
- Detailed analyses of several important allergens revealed a more heterogenous picture than expected



Glycan composition[a]	Identified by		Quant.
	MS [Da]	Acc. [ppm]	MS [%]
	1624.604*	3.7	31.4
	1462.552*	3.7	19.6
	2110.762*	2.7	13.9
	2255.800*	2.3	7.7
	1786.656*	2.7	7.5
	1234.436	2.2	2.9
	1259.465	1.0	2.0

Glycan composition[a]	Identified by		Quant.
	MS [Da]	Acc. [ppm]	MS [%]
	2475.888*	1.9	0.4
	2239.802	1.5	0.4
	1931.692	1.9	0.3
	2313.838	0.6	0.3
	2620.931*	1.5	0.3
	2637.945*	1.6	0.3
	1761.621	1.6	0.3

Wiegandt et al., Anal Chem 2014; Halim et al., Mol Cell Prot 2015

CCDs and allergenicity

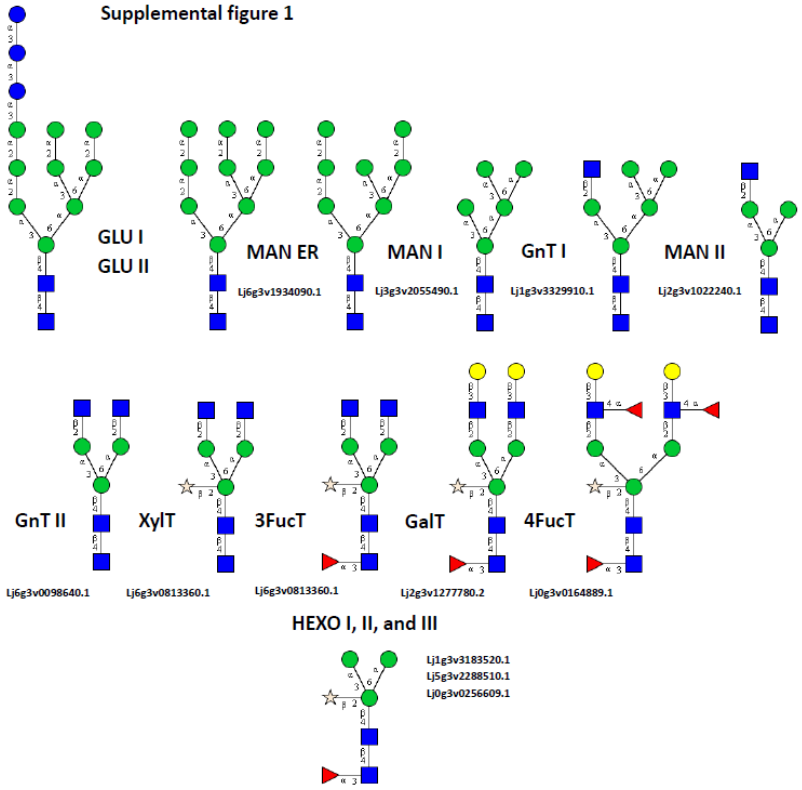
- Glycan structures such as fucose and xylose CCDs or alpha-Gal are panepitopes
- Affinity of IgE to CCDs can be extraordinarily high
- Frequently recognised also by normal individuals
- FX-CCD: (50% IgG) and allergic patients (30%)
- sIgE titers rise with AIT
- Probably the most frequently recognised IgE epitopes

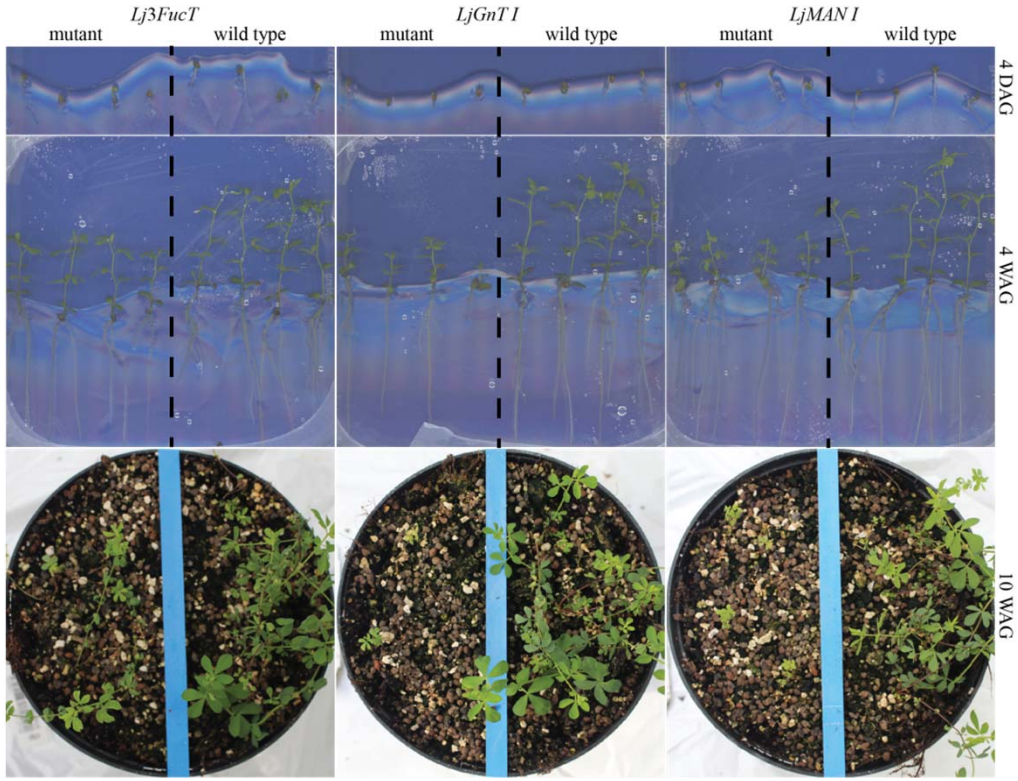
- Well documented clinical relevance of alpha-Gal and GOS
- No or limited clinical relevance of fx-CCDs

- **Questions:**
- Are CCDs biologically important?
- Are CCDs clinically relevant? If not, why not? If yes, how?
- Are CCD-specific IgE an exclusive, isolated phenomenon?
- Are CCD-specific IgE biologically active?

CCDs are biologically important modifications

- Biological function difficult to analyse, no obvious phenotype in Arabidopsis
- N-glycan mutant platform in *L. japonicus* using LORE1 retrotransposon
- Conserved pathway in Lotus but clear phenotype



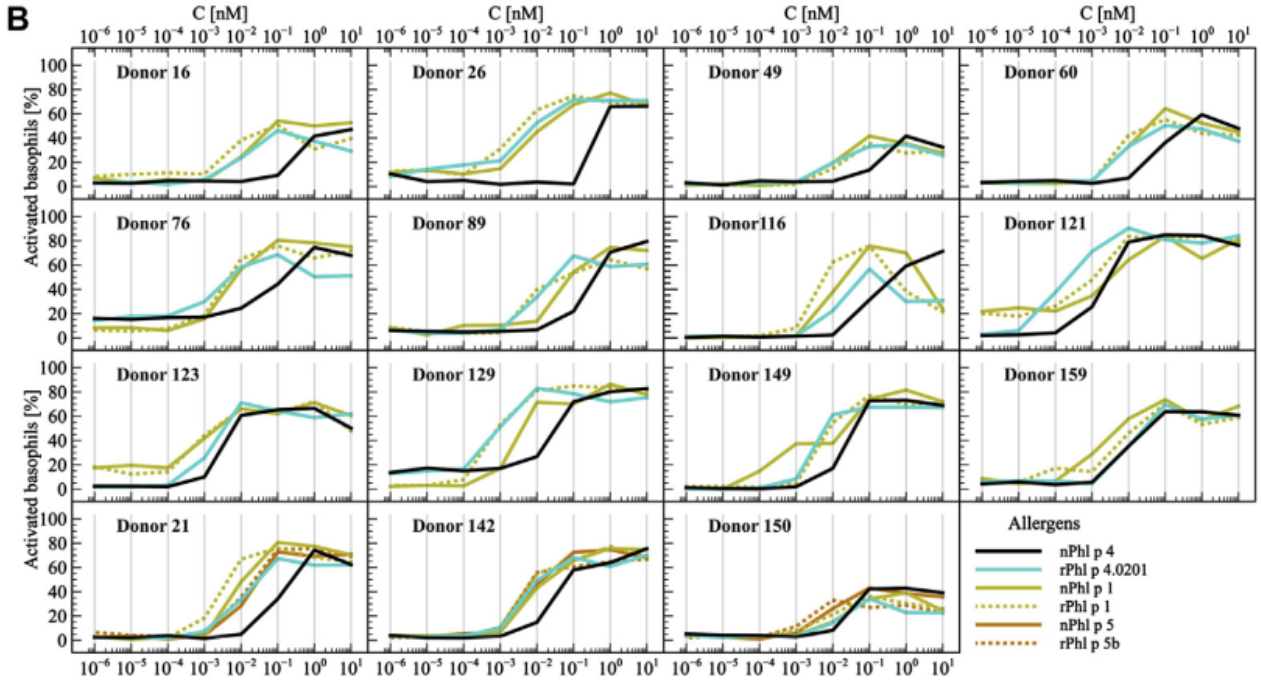


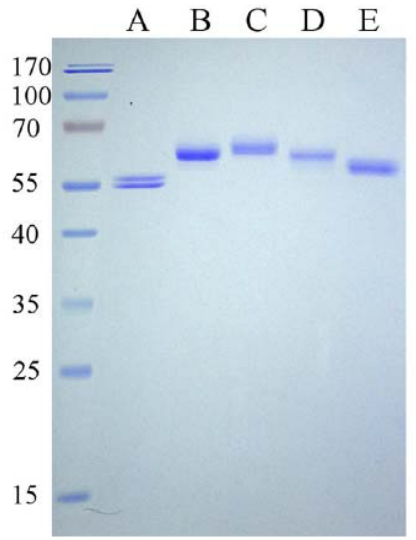
Pedersen et al., Plant J 2017

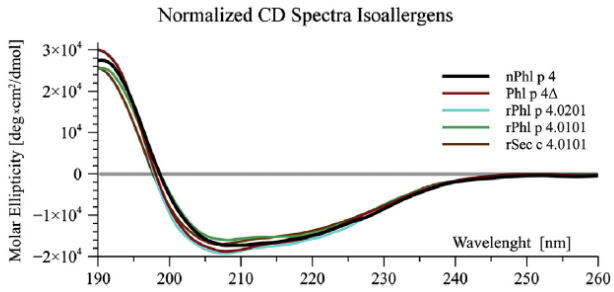
Glycans may reduce allergen activity

- Phl p 4: glycosylated allergen, clinical relevance discussed
- Expression of several variants of Phl p 4 in yeasts
- Reduced allergenic activity of natural Phl p 4

- Shielding of epitopes by glycans





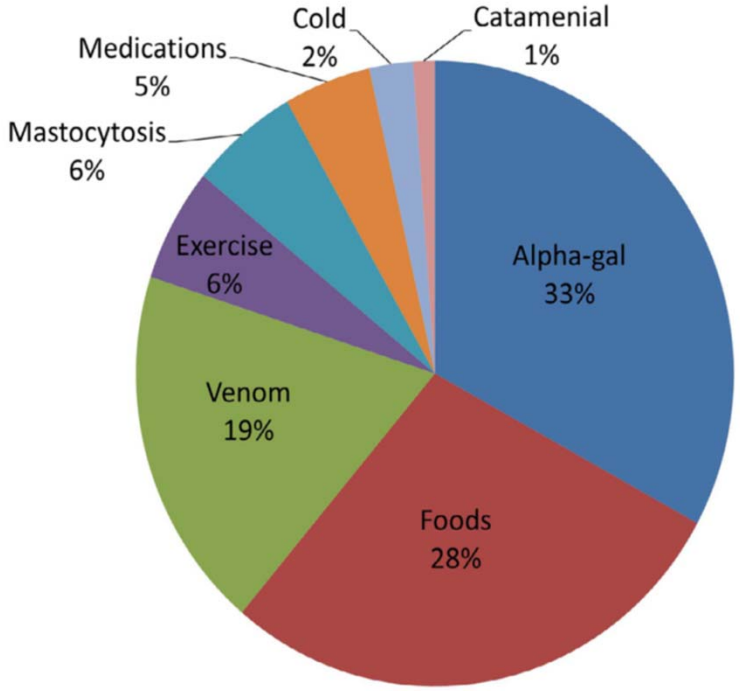


Zafred et al., J Allergy Clin Immunol 2014

Clinical relevance of CCDs

- FX-CCDs are reported to have limited capacity to induce symptoms in sensitized patients

- In contrast, α-Gal is well known for anaphylaxis to biologics and red meat allergy
- Retrospective study revealed number one culprit for anaphylaxis in defined areas



Pattanaik et al., Ann Allergy Asthma Immunol 2018

Allergenicity assessment beyond FX-CCDs

- Blocking with CCD-positive proteins such as bromelain, HRP or neoconjugates

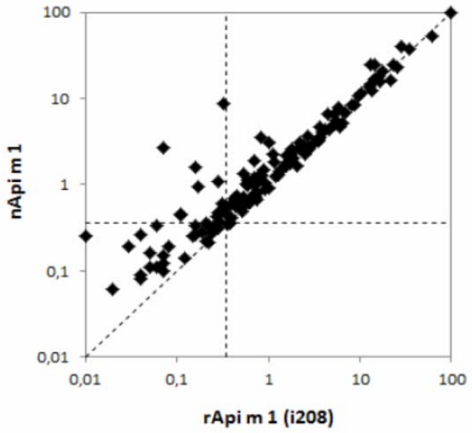
	Phadia CAP system		Mediwiss-Analytic AllergyScreen	
Allergen	kU/L usual test	kU/L with CCD-Blocker	kU/L usual test	kU/L with CCD-Blocker
Grasses	25	**1.1**	99	0.0
Birch	19.6	0.11	> 100	0.0
Ragweed	25.7	**0.49**	> 100	0.0
Mugwort	21.6	0.21	14.9	0.0
Hazelnut	17.4	0.08	16.5	0.0
Shrimp	2.6	0.12)	0.57	0.0
rec. Bet v 1	0.75	0.03 *)	-	-
Bee venom	28.5	1.1	-	-
Wasp venom	28.5	**18.3**	-	-
rApi m1	1.63	0.31	-	-
rVes v1	11.8	**9.7**	-	-
rVes v5	48.7	**44.6**	-	-

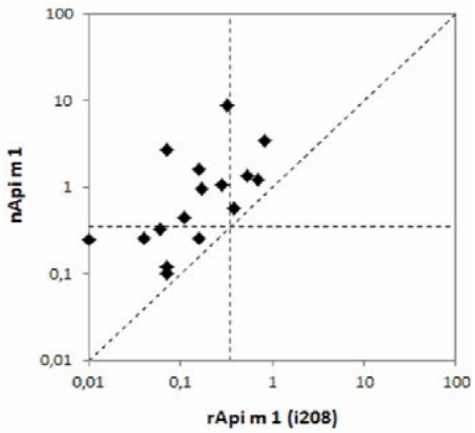
	ImmunoCAP (kU/L)		ISAC microarray (ISU-E)	
	Before CCD inhibition	After CCD inhibition	Before CCD inhibition	After CCD inhibition
Case 1				
Bromelain/MUXF^3-CCD	44.60	0.38	8.53	<0.3

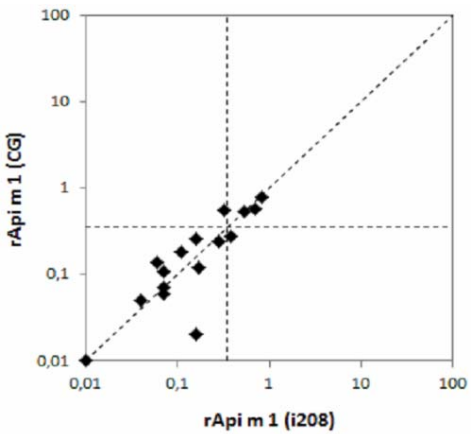
Hemmer et al., JACI 2018

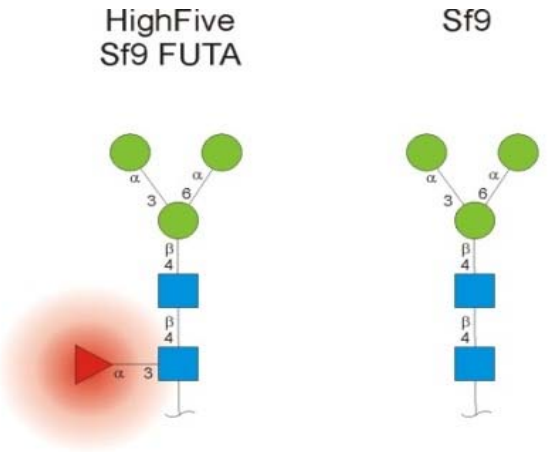
Recombinant, CCD-free allergens: Api m 1

- Lepidopteran Sf9 cells (*Spodoptera frugiperda*) and HighFive cells (*Trichoplusia ni*) have varying CCD phenotypes
- CCDs are absent in Sf9 cells due to absence of FucTX
- Glycan-free vs CCD-free, but glycoslated Api m 1









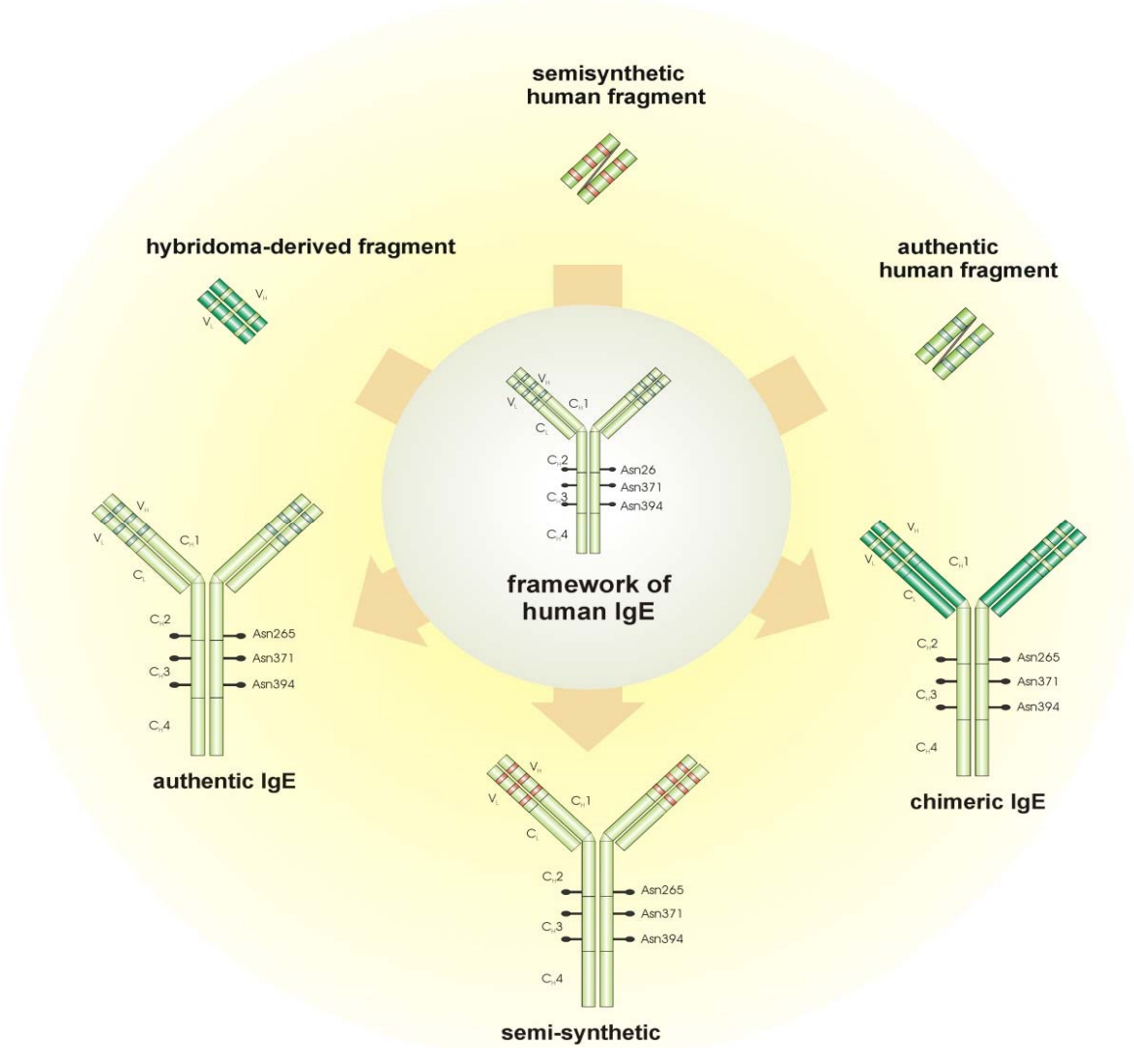
Seismann et al., Mol Immunol 2010, Jakob et al., J Allergy Clin Immunol 2012

Molecular analyses demand monoclonal IgE

- Establishment of recombinant IgE by different approaches

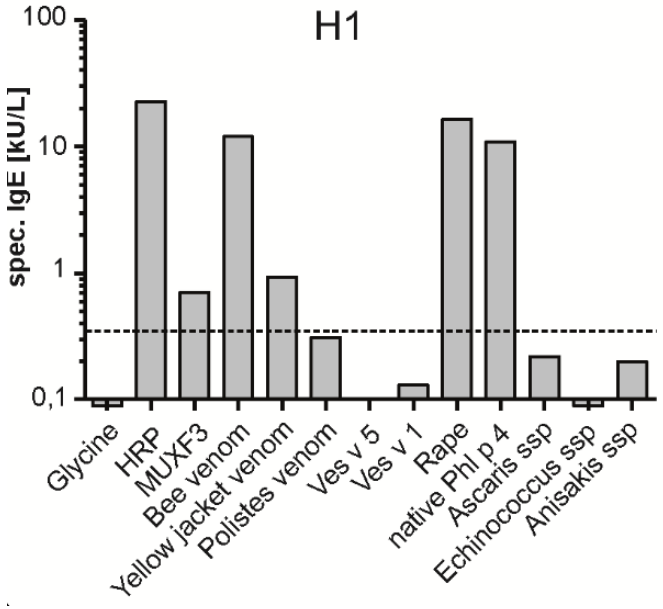
- Recloning of hybridomas
- Synthetic antibody repertoires
- Immune repertoires

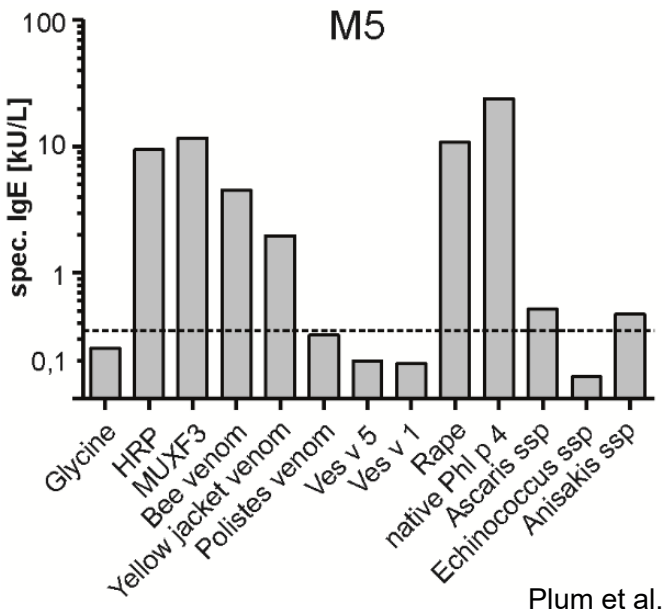
- Conversion to full antibody
- Production in suitable host cells



F-CCD and X-CCDs are recognised separately

- Selection of immune repertoire libraries yielded two major consensus sequences
- Conversion into IgE and production in mammalian and insect cells
- Analysis of interaction with antigen and downstream effects

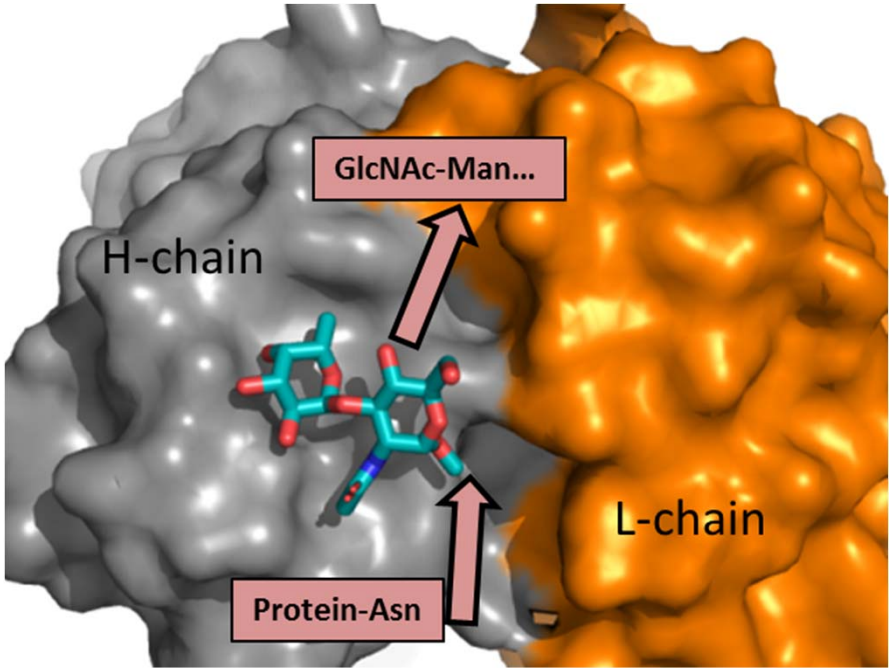


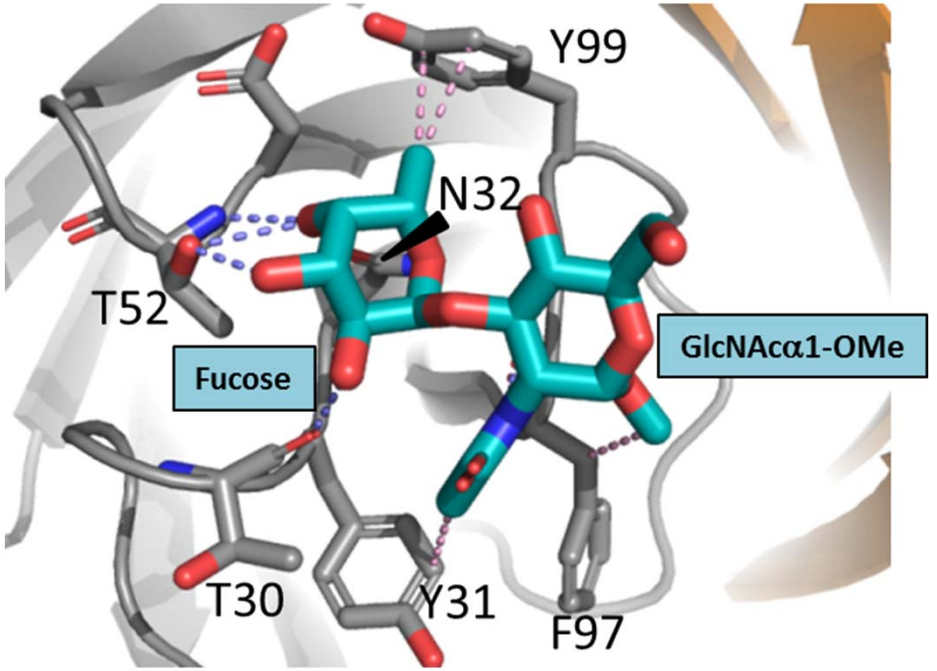


Plum et al., unpublished

Structural insights into CCD recognition

- Co-crystallisation of human IgG1 Fab with disaccharide epitope surrogate
- Emphasises the recognition mode of the core fucose

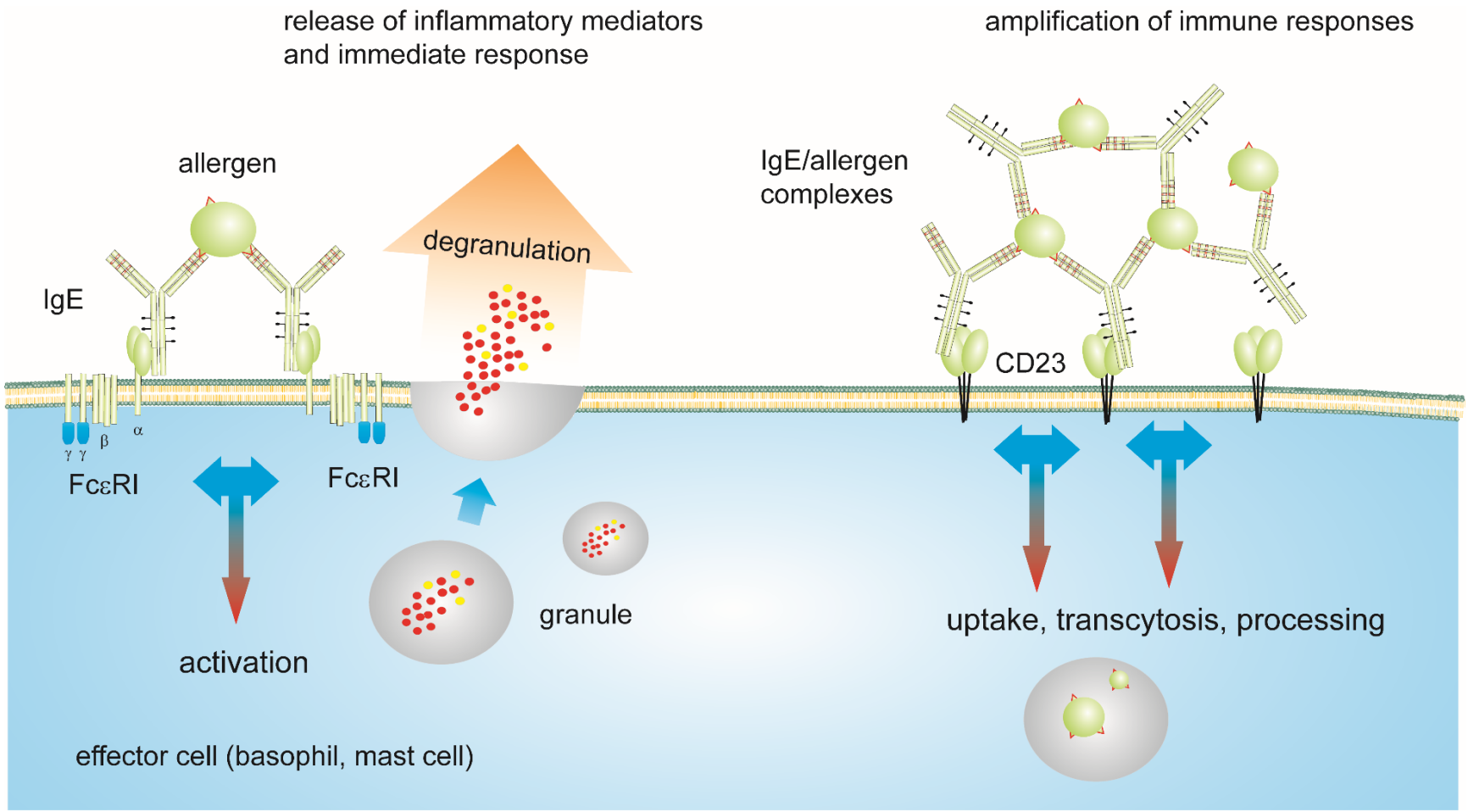




Plum et al., unpublished

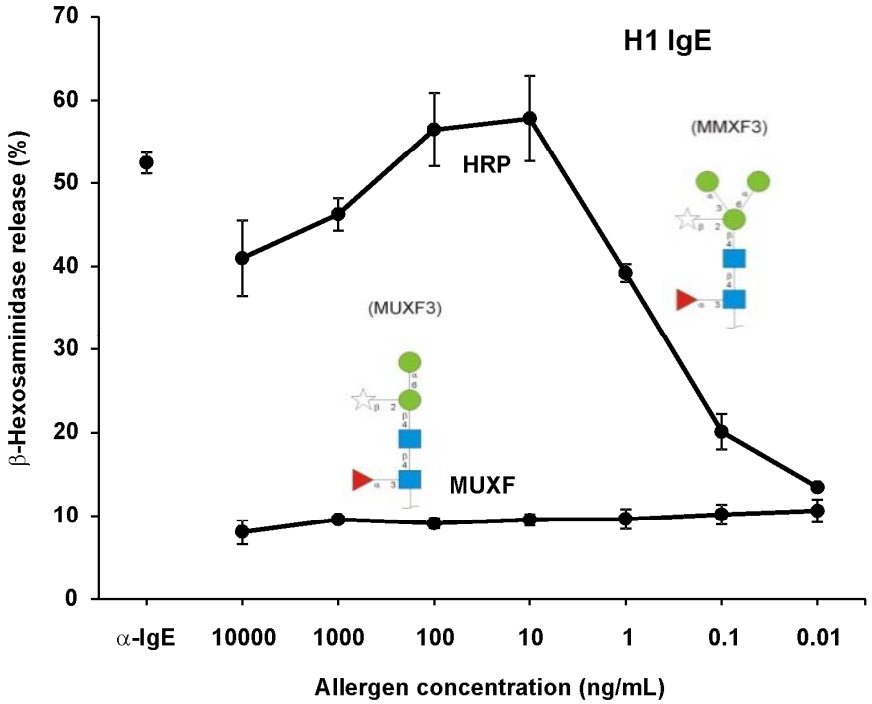
Functional activity of CCD-specific IgE

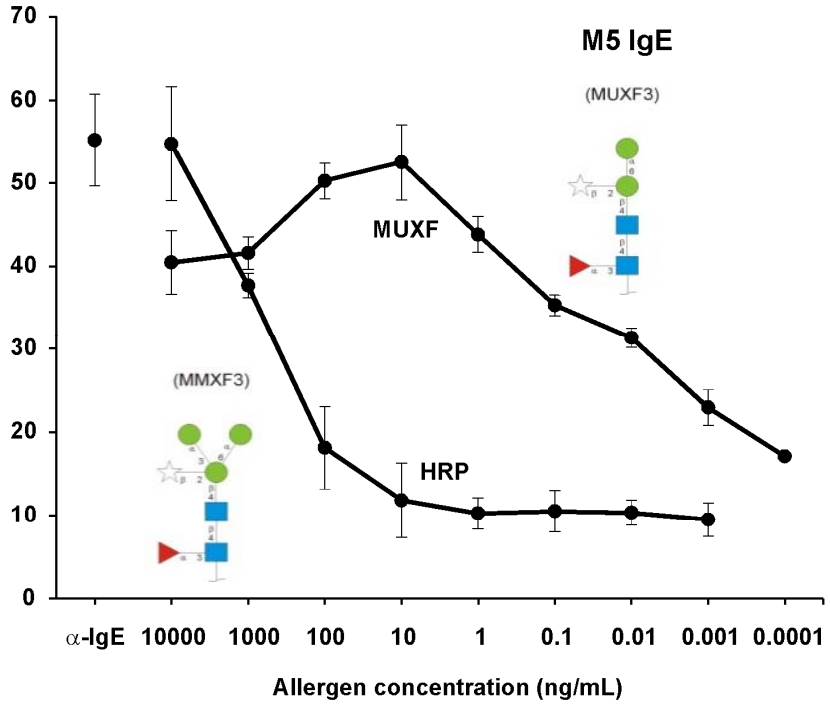
- Potential role within two major IgE axes as defined by the receptors



FcεRI-based activation by CCD-specific mIgE

- Pronounced degranulation of RBL SX38 by low amounts of glycoproteins
- Type of CCD (MUXF3 vs HRP glycan) determines activation

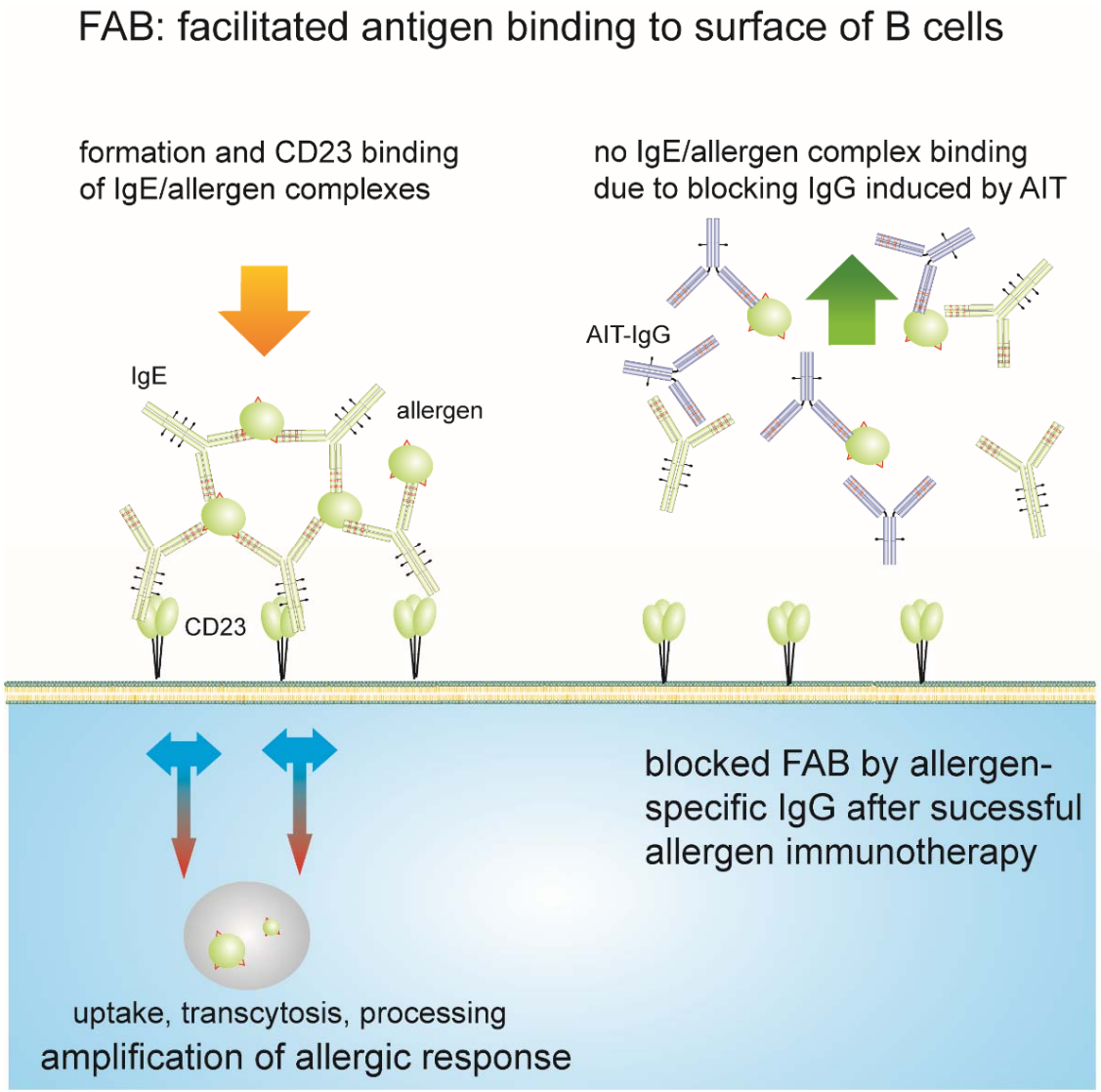




Plum et al., unpublished

Facilitated antigen binding via CD23

- FAB via binding of immune complexes
- Subsequent allergen presentation and T cell activation
- Detection by flow cytometry or solid phase assay
- Functional assessment of IgG4 induced by AIT
- Correlation with clinical picture



Current view on CCDs and allergenicity

- CCDs represent a single epitope only, comparison to allergens is apples and oranges
- Clear biological activity of CCDs and CCD-specific IgE
- Adds and contributes to the protein-directed IgE response
- Might contribute to shaping the IgE response by complex formation
- Not an exclusive reactivity, but part of an aberrant anti-carbohydrate response

- Information on presence and valency important
- Carbohydrate recognition could have adjuvant activity to modulate the immune response within allergic patients

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