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Biochemistry of pollen allergens

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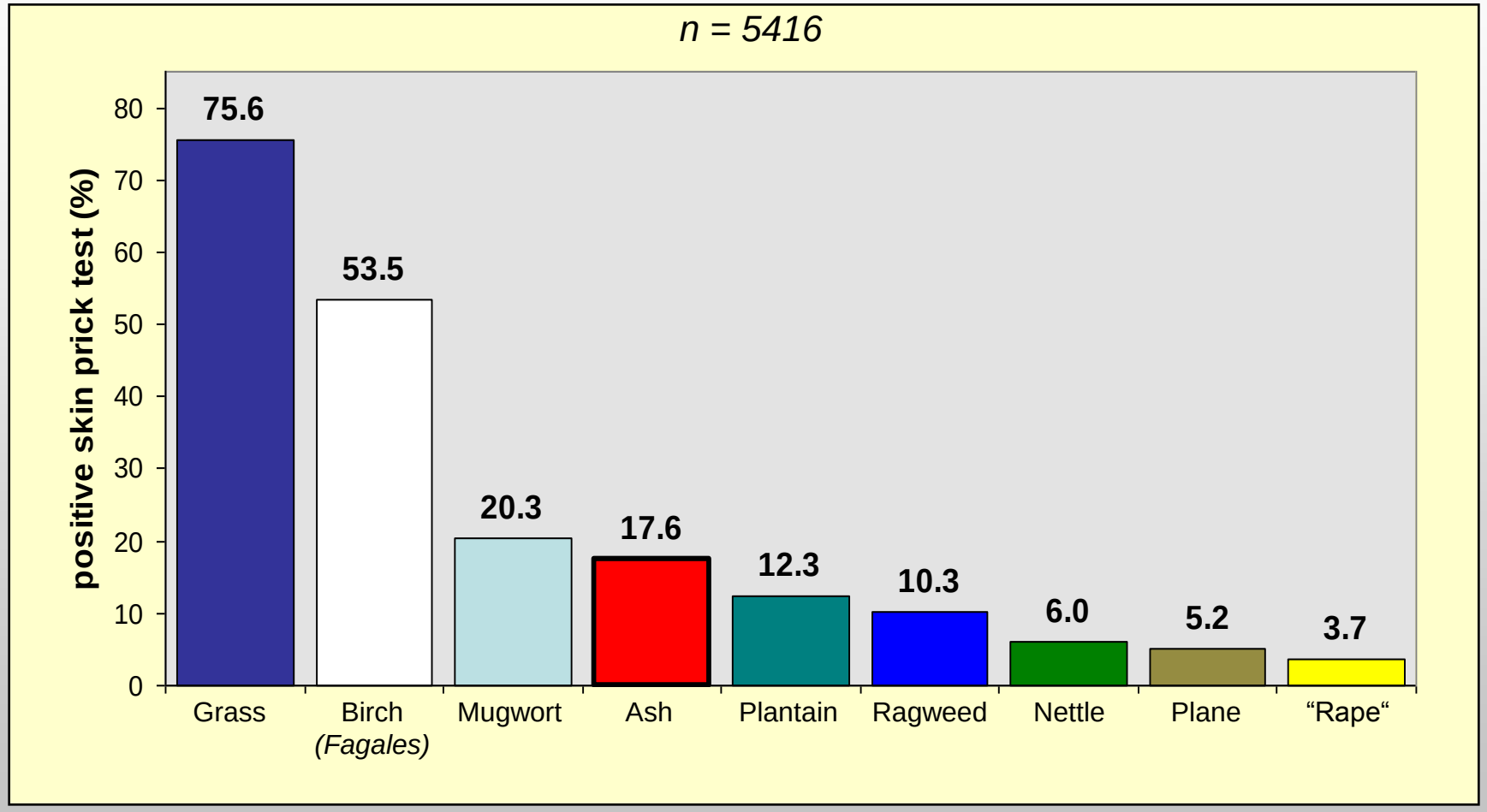
Overview

I. Relevant pollens and their allergens

II. Cross-reactive carbohydrate determinants

Prevalence of pollen allergens in Rakusko

(1997-1999)



Hemmer et al. (2000) Allergy 55

from the FAZ in Vienna

Allergenic pollens in Central Europe

1. Grasses

Timothygrass (Phleum pratense)
Ryegrass (Lolium perenne)
Rye (Secale cereale)
Red fescue (Festuca rubra)
Orchard grass (Dactylis glomerata)
Kentucky blue grass (Poa pratensis)

and other less related members of the Poaceae (grass family): e.g. Zea mays,

Allergenic pollens in Central Europe

Festuca

Poa

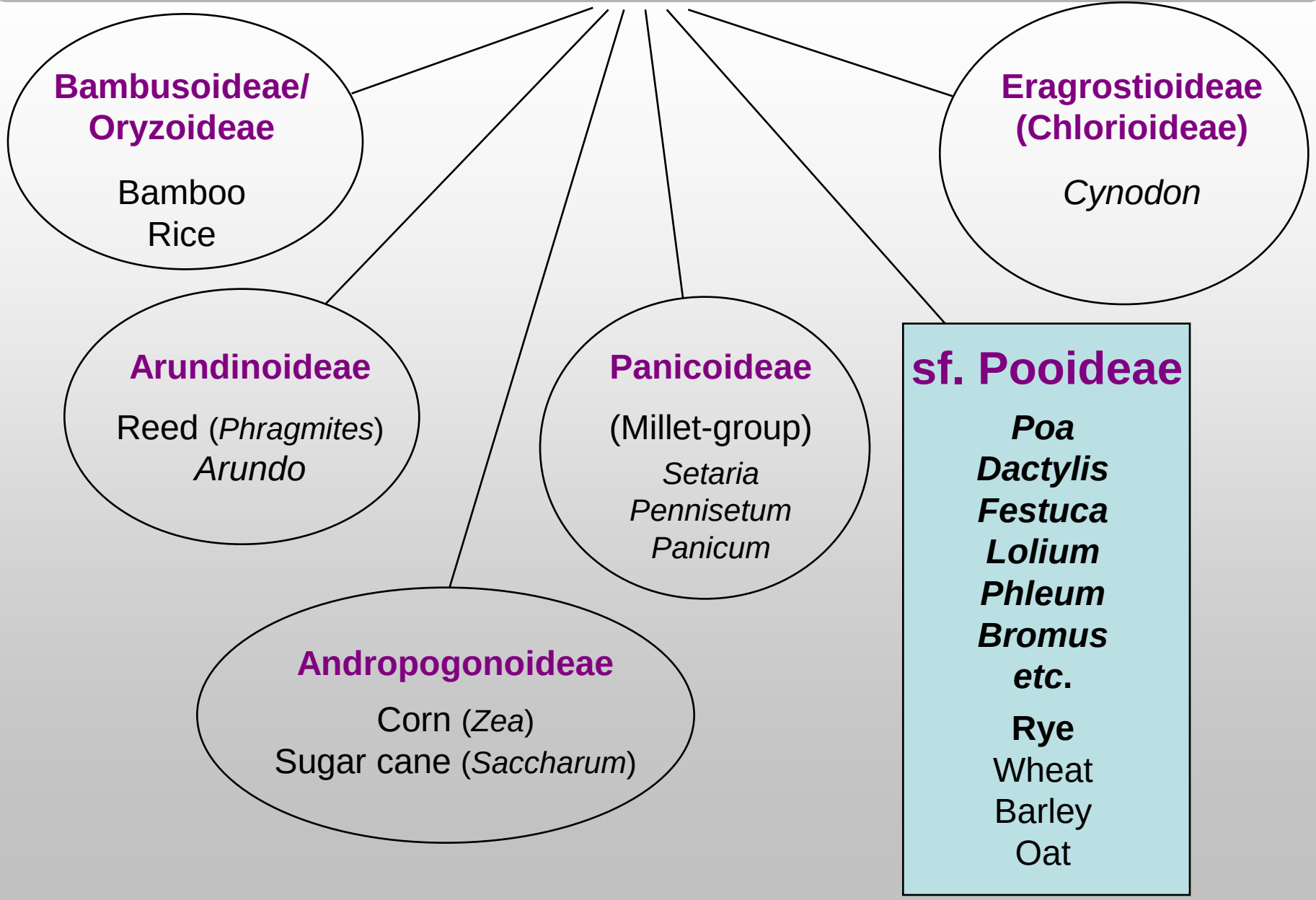
Dactylis

Phleum

Secale

Lolium

fam. Gramineae (Grasses)



Allergenic pollens in Central Europe

2. **Birch** (Betula verrucosa) and related trees

alder
(Alnus glutinosa)

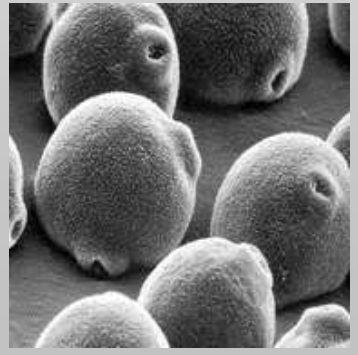
hornbeam
(Carpinus betulus)

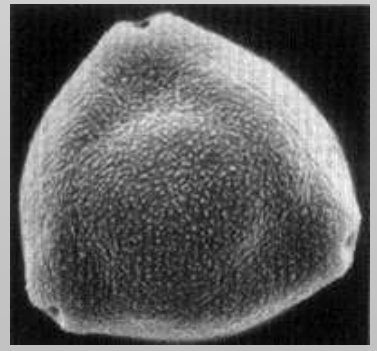
hazel
(Corylus avellana)

Allergenic pollens in Central Europe

Fagales

Betulaceae	Corylaceae	Fagaceae
Birch (*Betula*)	Hazel (*Corylus*)	Oak (*Quercus*)
Alder (*Alnus*)	Hornbeam (*Carpinus*)	Beech (*Fagus*)
		ed. Chestnut (*Castanea*)





Allergenic pollens in Central Europe

3. **Mugwort** (Artemisia vulgaris)

Most important weed pollen

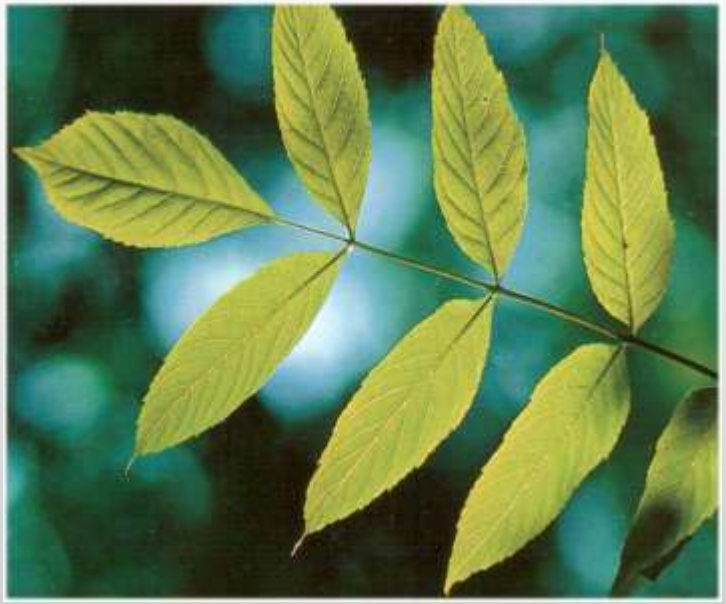
up to 20 % of pollen allergic pts.

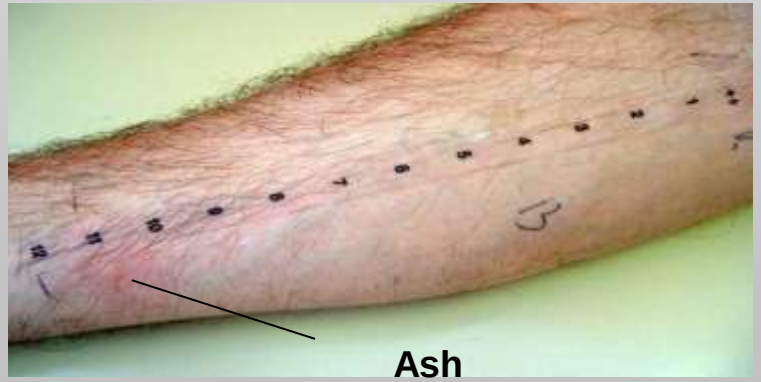
Allergenic pollens in Central Europe

4. Ash (Fraxinus excelsior)

often „real allergy“

sometimes
isolated sensitization





Ash pollen

Allergenic pollens in Central Europe

5. Short **Ragweed** (Ambrosia artemisiifolia)

in ca 75 % pats. associated with mugwort sensitization

form USA,
spreading from Hungary since ca. 1910
sensitizations increasing

Allergenic pollens in Central Europe

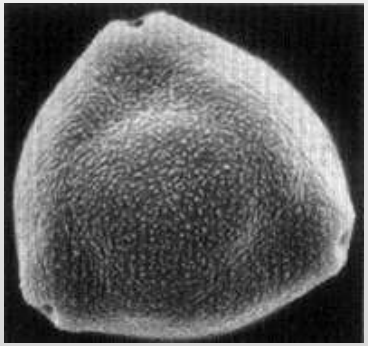
furthermore:

6. **Nettle** (Urtica dioeca) (cross-reactivity with Parietaria judaica)
7. **Plantain** (Plantago lanceolata) / **Olive** (Olea europaea)
8. **Plane-tree (Sycamore)** (Platanus acerifolia)

and others less important or just cross-reactive pollens
Gymnosperms (cypress) in Central Europe irrelevant

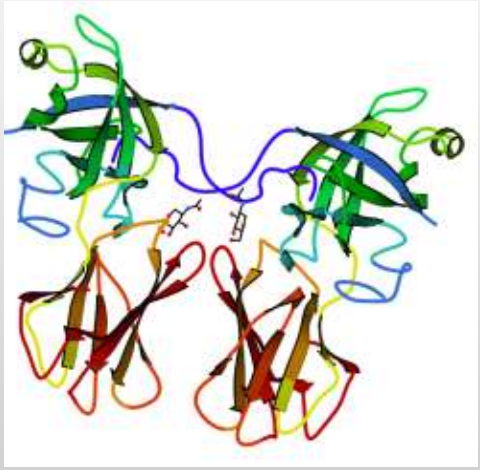
What is an allergen ?

Allergen



Allergen source

Allergen

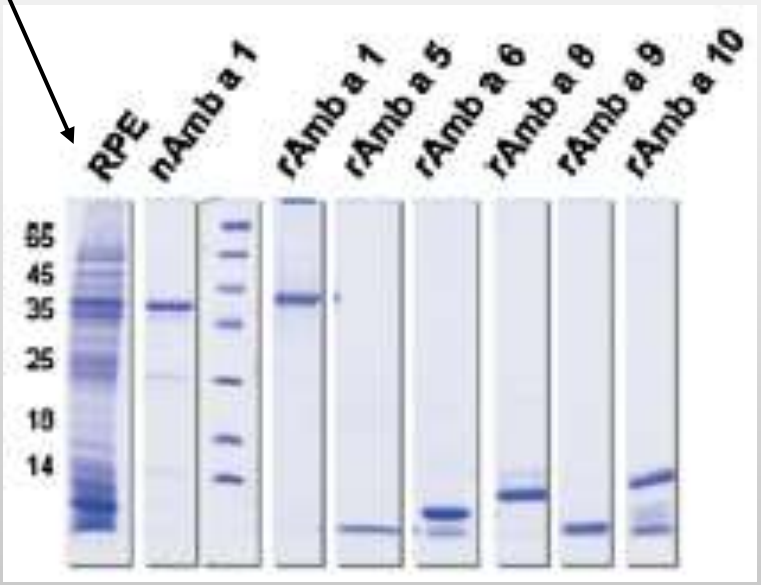


Identification of allergens

Ragweed pollen extract

Recombinant Ragweed allergens

Ragweed pollen

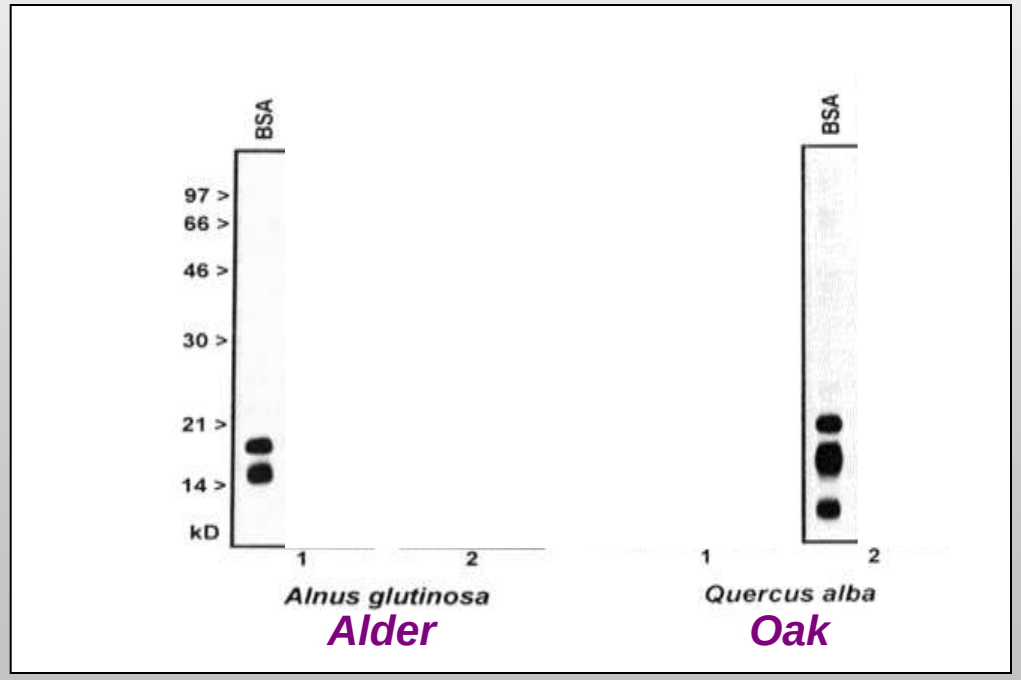


from Chapman et al.
2006 JACI

Identification of allergens

by immunoblotting

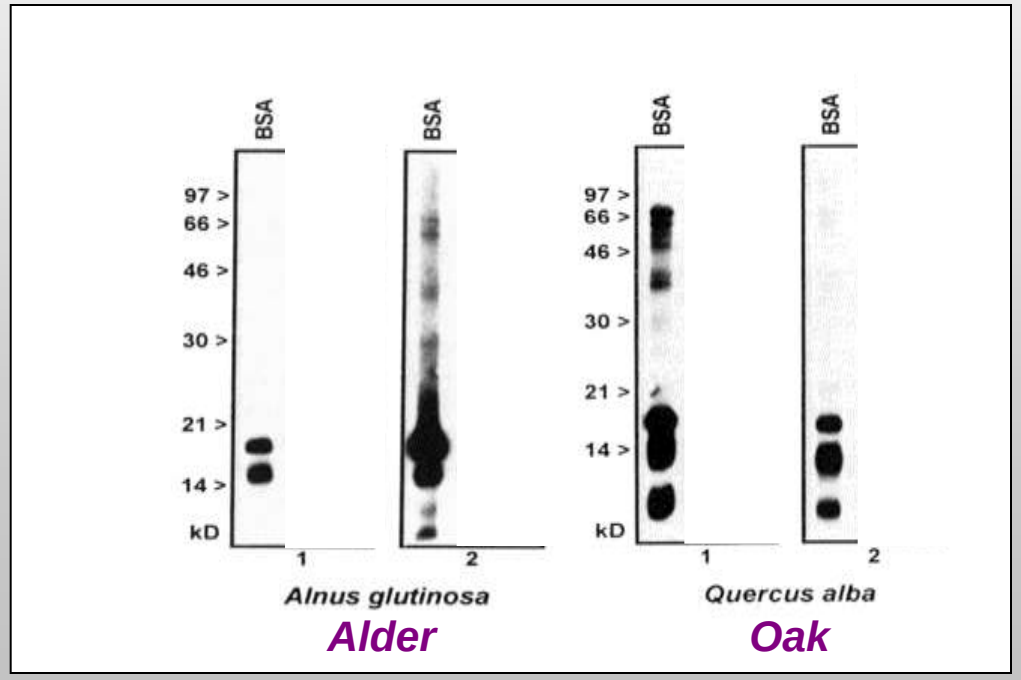
extract of allergen – SDS-PAGE – patient serum
detection of bound IgE by labeled anti-IgE



Cross-reactivity of allergens

Oak-pollen sensitized patient reacts with alder pollen and *vice verca*

mere conincidence ?

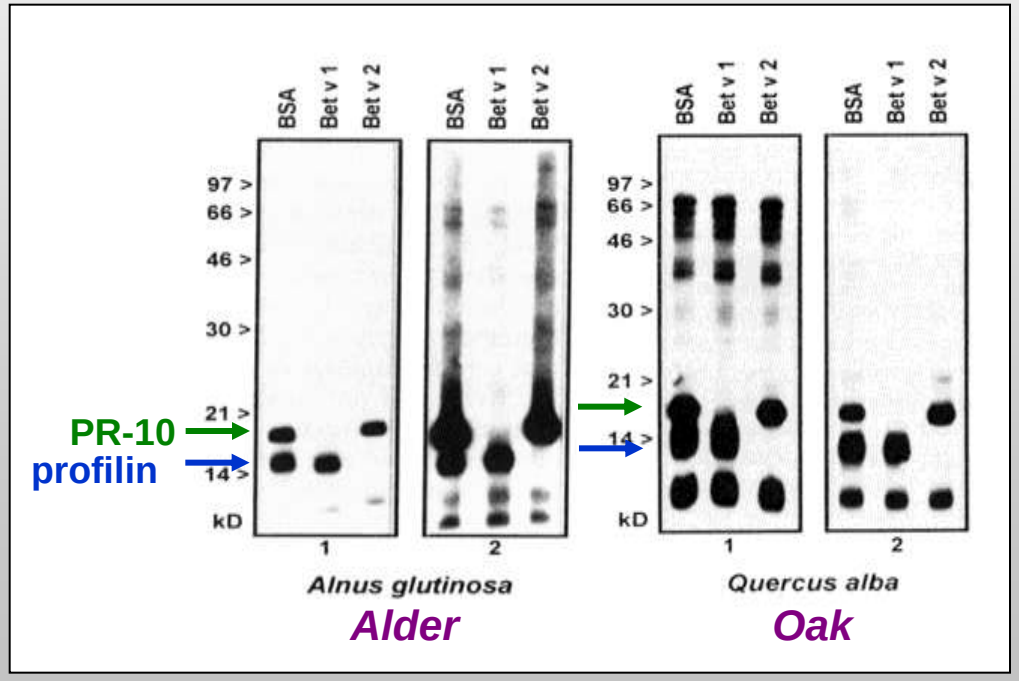


Cross-reactivity of allergens

specific inhibition of interaction by pure birch pollen allergens

Bet v 1 …. PR10 defense related protein

Bet v 2 …. profilin



Cross-reactivity of allergens

Bet v 1 (PR10 protein) homologous to Aln g 1, Cor a 1, Car b 1
and also Mal d 1, Pru av 1 and other food allergens (> 75 % 1)

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                1       10        20        30        40        50        60        70        80        90       100       110       120       130
                |-------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
BETV1A          MGVFNYETETTSVIPAARLFKAFILDGDNLFPKVAPQAISSVENIEGNGGPGTIKKISFPEGFPFKYVKDRVDEVDHTNFKYNYSVIEGGPIGDTLEKISNEIKIVATPDGGSILKISNKYHTKGDHEVK
CORAV1          MGVFNYETESTSVIPAARLFKAFILDGNNLIPKVAPQAVSSVENVEGNGGPGTIKKITFSEGSPFKYVKERVEEVDHTNFKYSYTVIEGGPVGDKVEKICNEIKIVAAPDGGSILKISNKYHTKGDHEVD
ALNGL            MGVFNYEAETPSVIPAARLFKAFILDGDKLLPKVAPEAVSSVENIEGNGGPGTIKKITFPEGSPFKYVKERVDEVDRVNFKYSFSVIEGGAVGDALEKVCNEIKIVAAPDGGSILKISNKFHTKGDHEIN
CARB1           MGVFNYEAETPSVIPAARLFKSYVLDFDKLIPKVAPQAISSVENVGGNGGPGTIKNITFAEGSPFKFVKERVDEVDHANFKYNYTVIEGDVLGDKLEKVSHELKIVAAPGGGSIVKISSKFHAKGDHEVN
Consensus       MGVFNYEaEtpSVIPAARLFKa%!LDg#kLiPKVAP#A!SSVEN!eGNGGPGTIKkItF.EGsPFK%VK#RV#EVD..NFKYn%tVIEGg..GDklEK!snEiKIVAaPdGGSIlKISnK%HtKGDHE!n

                131     140       150       160
                |-------+---------+---------|
BETV1A          AEQVKASKEMGETLLRAVESYLLAHSDAYN
CORAV1          AEHIKGGKEKVEGLFRAVEAYLLAHSDAYN
ALNGL            AEQIKIEKEKAVGLLKAVESYLLAHSDAYN
CARB1           AEKMKGAKEMAEKLLRAVESYLLAHTDEYN
Consensus       AE..Kg.KEmae.LlrAVEsYLLAHsDaYN
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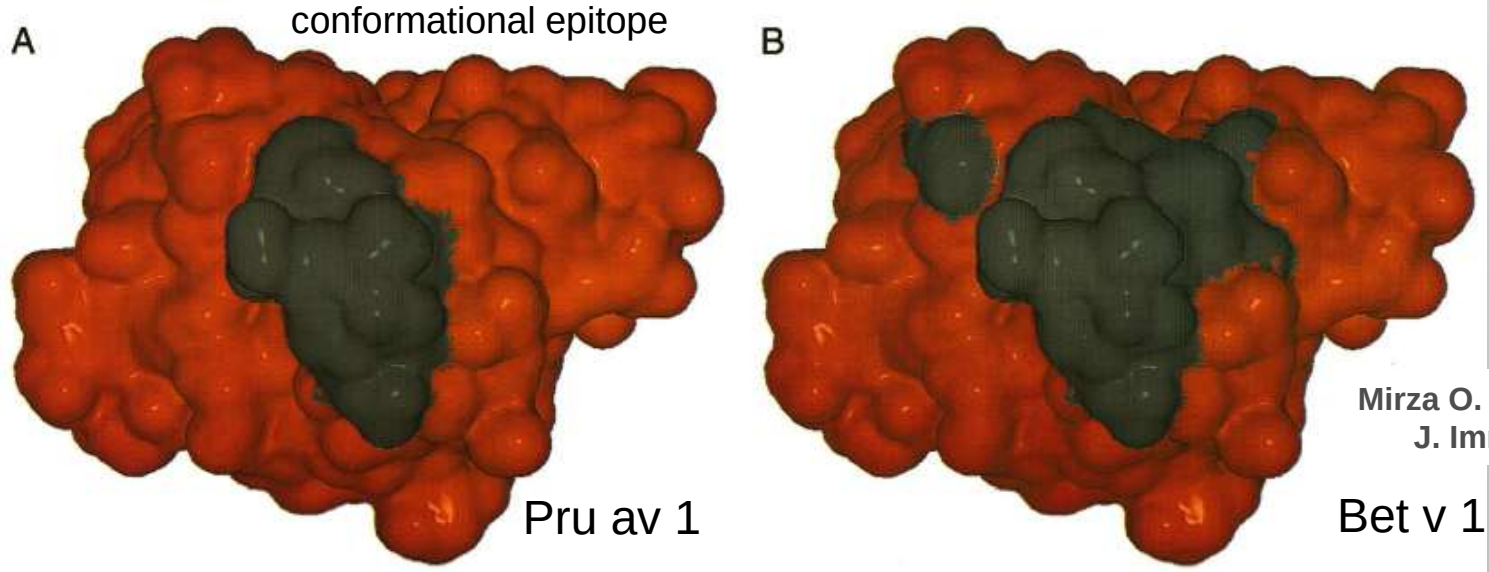
Structural basis for cross-reactivity between birch, alder, hornbeam, hazelnut
or oak pollen and also food allergens in e.g. apples, cherries, peach (even soya)



Mirza O. et al (2000)
J. Immunol.

Cross-reactivity of allergens

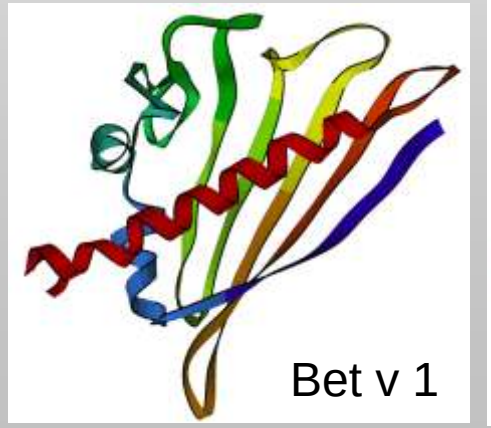
Bet v 1 comes in many isoforms (alleles from one gene locus or paralogs)

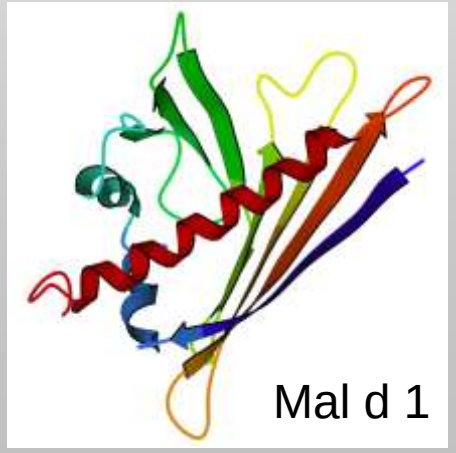
Bet v 1.0101	Bet v 1.0102	Bet v 1.0103	Bet v 1.0201	Bet v 1.0301	Bet v 1.0401	Bet v 1.0402	Bet v 1.0501
Bet v 1.0601	Bet v 1.0602	Bet v 1.0701	Bet v 1.0801	Bet v 1.0901	Bet v 1.1001	Bet v 1.1101	Bet v 1.1201
Bet v 1.1301	Bet v 1.1401	Bet v 1.1402	Bet v 1.1501	Bet v 1.1502	Bet v 1.1601	Bet v 1.1701	Bet v 1.1801
Bet v 1.1901	Bet v 1.2001	Bet v 1.2101	Bet v 1.2201	Bet v 1.2301	Bet v 1.2401	Bet v 1.2501	Bet v 1.2601
Bet v 1.2701	Bet v 1.2801	Bet v 1.2901	Bet v 1.3001				

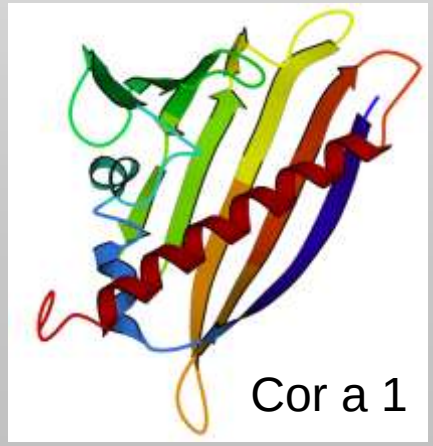
from the Allergen Nomenclature Sub-Committee (www.allergen.org)

“hypoallergenic“ isoforms or derivatives

Bet v 1 has many “orthologs“ in other species





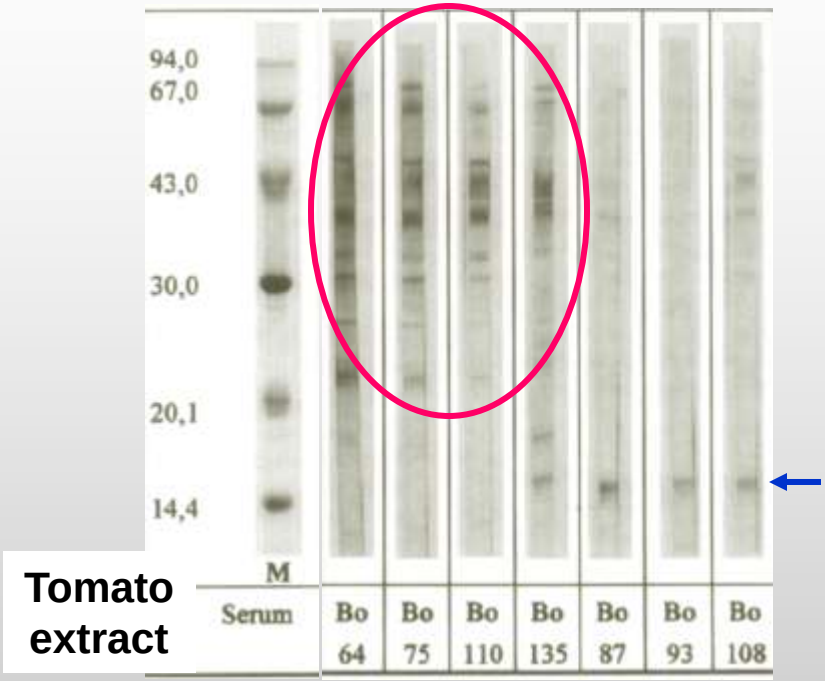


Nomenclature !!!

Bet v 2 (profilin) homologs in

Birch pollen	Bet v 2
Mugwort pollen	Art v 2
Pellitory pollen	Par j 3
Timothy pollen	Phl p 12
Apple	Mal d 4
Beach	Pru p 4
Latex milk	Hev b 8
Soy milk	Gly m 3
Tomato	Lyc e 1
…..	

from Petersen A. et al (1996) JACI 98

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Bet             MSWQTYVDEHLMCDIDGQASNSLASAIVGHDGSVWAQSSSFPQFKPQEITGIMKDFEEPGHLAPTGLHLGGIKYMVIQGEAGAVIRGKKGSGGITIKKTGQALVFGIYEEPVTPGQCNMVVERLGDYLID
Mal             MSWQAYVDDHLMCDIDGH--HLTAAAILGHDGSVWAQSSTFPKFKPEEITAIMKDFDEPGSLAPTGLHLGGTKYMVIQGEGGAVIRGKKGSGGVTVKKTGQDLVFGIYEEPLTPGQCNMIVERLGDYLID
Hev             MSWQTYVDEHLMCDIDGH--HLTAAAIIGHDGSVWAQSSSFPQFKPEEVAAIMKDFDEPGSLAPTGLHLGGTKYMVIQGEPGAVIRGKKGSGGITVKKTGQALIIGIYDEPLTPGQCNMIVERLGDYLLE
Phl             MSWQTYVDEHLMCEIEGH--HLASAAILGHDGTVWAQSADFPQFKPEEITGIMKDFDEPGHLAPTGMFVAGAKYMVIQGEPGRVIRGKKGAGGITIKKTGQALVVGIYDEPMTPGQCNMVVERLGDYLVE
Pru             MSWQAYVDDHLMCEIEGN--HLSAAAIIGHDGSVWAQSATFPQLKPEEVTGILNDFNEPGSLAPTGLYLGGTKYMVIQGEPGAVIRGKKGPGGVTVKKSTLALLIGIYDEPMTPGQCNMIVERLGDYLVE
Gly             MSWQAYVDDHLLCDIEGN--HLTHAAIIGQDGSVWAQSTDFPQFKPEEITAIMNDFNEPGSLAPTGLYLGGTKYMVIQGEPGAVIRGKKGPGGVTVKKTGAALIIGIYDEPMTPGQCNMVVERPGDYLID
Lyc             MSWQTYVDEHLLCENEGN--HLTSAAIIGQDGTVWAQSANFPQFKPEEITGIMNDFAVPGTLAPTGLYLGGTKYMVIQGEPEAVIRGKKGPGGITIKKTNQALIIGIYDEPMTPGQCNMIVERLGDYLIE
Par             MSWQAYVDDHLMCDV-GDGNTPASAAIIGHDGSVWAQSANFPQLKPEEVTGIMNDFNEAGFLAPTGLFLGGTKYMVIQGESGAVIRGKKGSGGATLKKTGQAIVIGIYDEPMTPGQCNLVVERLGDYLLE
Art             MSWQTYVDDHLMCDIEGTGQHLTSAAIFGTDGTVWAKSASFPEFKPNEIDAIIKEFNEAGQLAPTGLFLGGAKYMVIQGEAGAVIRGKKGAGGICIKKTGQAMVFGIYDEPVAPGQCNMVVERLGDYLLD
Consensus       MSWQtYVD#HL$C#ieG...hltsaAIiGhDGsVWAqSa.FP#fKP#E!tgImk#F.epG.LAPTG$.lgGtKYMVIQGEpgaVIRGKKG.GGit.KKtgqAlviGIY#EPmtPGQCN$!VERlGDYL.#
```

sequences from http://au.expasy.org/uniprot
alignment by Multalign http://bioinfo.genopole-toulouse.prd.fr/multalin/multalin.html

IgE-binding and allergy

high affinity
polyvalent
binding

Sensitization

IgE binding to allergen

measured by

- RAST, CAP etc.
- immunoblot, allergen array

danger of false positives

Allergy

Clinical symptoms upon contact with allergen

measured by

- Skin prick test (SPT)
- Allergen exposure

Grass pollen allergens

How to diagnose the right grass pollen allergy ?

Poa pratensis, Festuca rubra, Phleum pratense,
Dactylis glomerata, Arrhenaterum elatius, Lolium perenne,
Holcus lanatus, Agrostis stolonifera, Anthoxanthum odoratum,
Alopecuris pratensis, Secale cereale, Triticum vulgare,
Hordeum vulgare, Avena sativa, Zea mays,
Phragmites communis, and *Cynodon dactylon*.

Grass pollen allergens

Source of (too much) information:

Allergome database (allergome.org)

Allergen nomenclature website (allergen.org)

Example: Phleum – timothy grass

Allergen	Biochemical name	MW(SDS_PAGE)
Phl p 1	Expansin	27
Phl p 2	Grass group II/III	10-12
Phl p 4		55
Phl p 5	Ribonuclease	32
Phl p 6		11
Phl p 7	Calcium binding protein	6 (8.6 kD calc mass)
Phl p 11	Trypsin Inhibitor	20
Phl p 12	Profilin	14
Phl p 13	Polygalacturonase	55

Grass pollen allergens

Allergological significance of Phleum allergens

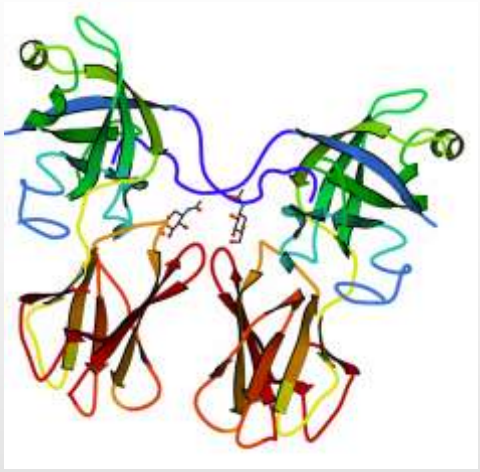
Phl p 1	95 % of grass pollen allergic patients
Phl p 2	65 % IgE binding
Phl p 4	55 kDa, many positives, but probably glycoprotein (see later !)
Phl p 5	95 % of grass pollen allergic patients
Phl p 6	75 % of patients, histamine, skin reactions
Phl p 7	10 %
Phl p 11	32 %
Phl p 12 (profilin)	ca. 75 %, relevant ?
Phl p 13	55 KDa, glycoprotein

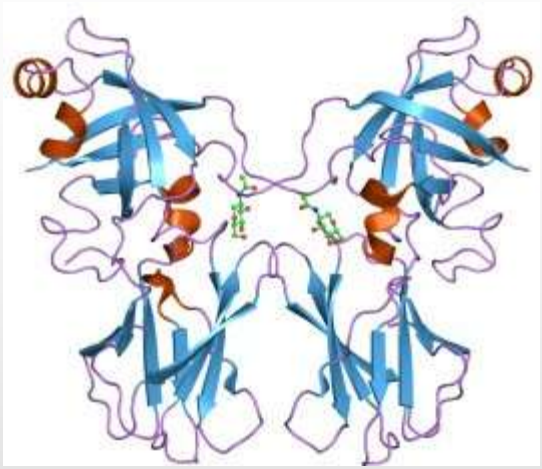
Grass pollen allergens

Phl p 1

high homology with
other group I allergens
from Dactylis, Poa, Lolium

Sequence ↔ Structure





Grass pollen group 1 allergens … expansins

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Phlp1            MASSSSVLLVYVLFAVFLGSAYGIPKVPPGPNITATYGDKWLDAKSTWYGKPTGAGPKDNGGACGYKDVDKPPFSGMTGCGNTPIFKSGRGCGSCFEIKCTKPEACSGEPVYVHITDDNEEPIAPYHFD
Dacg1           MASSSSSVLLVVALFAVFLGSAHGIPKVPPGPNITATYGDKWLDAKSTWYGKPTGAGPKDNGGACGYKDVDKAPFNGMTGCGNTPIFKDGRGCGSCFEIKCTKPESCSGEAVTVHITDDNEEPIAPYHFD
Poap1            MASSSSVLLVVALFAVFLGTAHGIAKVPPGPNITATYGDKWLDAKSTWYGKPTGAGPKDNGGACGYKDVDKAPFSGMTGCGNTPIFKSGRGCGSCFEIKCTKPESCSGEPVLVHITDDNEEPIAAYHFD
Lolp1            MASSSSVLLVVALFAVFLGSAHGIAKVPPGPNITAEYGDKWLDAKSTWYGKPTGAGPKDNGGACGYKNVDKAPFNGMTGCGNTPIFKDGRGCGSCFEIKCTKPESCSGEAVTVTITDDNEEPIAPYHFD
Consensus       .maSSSSVLLVVaLFAVFLGsAhGIaKVPPGPNITAtYGDKWLDAKSTWYGKPTGAGPKDNGGACGYK#VDKaPFnGMTGCGNTPIFKdGRGCGSCFEIKCTKPEsCSGEaVtVhITDDNEEPIApYHFD

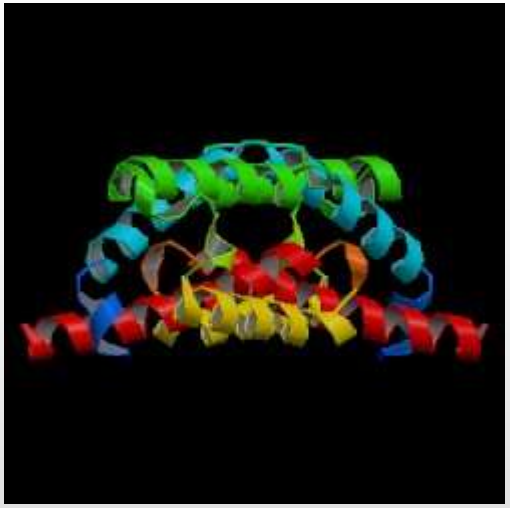
                131     140       150       160       170       180       190       200       210       220       230       240       250       260
                |-------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Phlp1           LSGHAFGAMAKKGDEQKLRSAGELELQFRRVKCKYPEGTKVTFHVEKGSNPNYLALLVKYVNGDGDVVAVDIKEKGKDKWIELKESWGAIWRIDTPDKLTGPFTVRYTTEGGTKTEAEDVIPEGWKADTS
Dacg1           LSGHAFGSMAKKGEEQKLRSAGELELQFRRVKCKYPEGTKVTFHVEKGSNPNYLALLVKYVDGDGDVVAVDIKEKGKDKWIALKESWGAIWRVDTPDKLTGPFTVRYTTEGGTKSEVEDVIPEGWKADTS
Poap1           LSGKAFGAMAKKGEEQKLRSAGELELKFRRVKCEYPEGTKVTFHVEKGSNPNYLALLVKYVTGDGDVVAVDIKEKGKDKWIELKESWGSIWRVDTPDKLTGPFTVRYTTEGGTKGEAEDVIPEGWKADTA
Lolp1           LSGHAFGSMAKKGEEQNVRSAGELELQFRRVKCKYPDDTKPTFHVEKASNPNYLAILVKYVDGDGDVVAVDIKEKGKDKWIELKESWGAVWRIDTPDKLTGPFTVRYTTEGGTKSEFEDVIPEGWKADTS
Consensus       LSGhAFGsMAKKG#EQkLRSAGELELqFRRVKCkYP#gTKvTFHVEKgSNPNYLAILVKYVdGDGDVVAVDIKEKGKDKWIeLKESWGa!WR!DTPDKLTGPFTVRYTTEGGTKsE.EDVIPEGWKADTs

                284
                |--|
Phlp1           YESK
Dacg1           YEAK
Poap1           YASK
Lolp1           YSAK
Consensus       Y.aK
```

Grass pollen allergens

Phl p 5

also quite high homology



Grass pollen group 5 allergens … ribonucleases

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           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Phlp5            AYPRRGPRGGPGRSYAADAGYAPATPAAAGAEAGKAT-------------------TEEQKLIEDINVGFKAAYAAAASVPAGDKFKTFEAAF-TSSSKAATA-----KAPG----LVPKL
Dacg       MAVQKYTVALFLAVVLVAGPVASYAADAGYTPAAAATPATAGGKAM-------------------TEEQTLIEDVNAGFKAAYAAASSAPPADKFKTFEATF-TAACKANIAAA-ATKVPL----FVAKL
Poap5      MAVQKYTVALFLTVALVAGPAASYAADAGYAPATPAAAGAAAGKITP-------------------TQEQKLMEDINVGFKAAYAAAAGAPPADKFKTFQAAF-SASVEASAAKLNAAQAPG----FVSHV
Lolp5b     MAVQKHTVALFLAVALVAGPAASYAADAGYAPATPATPAAPATAATPATPATPATPAAVPSGKATTEEQKLIEKINAGFKAAYAAAAVVPPADKYKTFVETFGTATNKAFVEGLASGYADQSKNQLTSKL
Consensus  mavqk.tvalflavalvaGPaaSYAADAGYaPAtpAtpaa.agkatp.................T#EQkLiEd!NaGFKAAYAAAAa.vPpaDK%KTF.atF.ta..kA..a.l....ap.....lvskl

           131     140       150       160       170       180       190       200       210       220       230       240       250       260
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Phlp5      DAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEVHAVKPVTEEPGMAKIPAGELQIIDKIDAAFKVAATAAATAPADDKFTVFEAAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAATVAAAP
Dacg       DAAYAVAYKTATGPTPEAKYDAFVAALTEALRVIAGALEVHAVKPAAEEVPAAKIPAGELQIVDKIDAAYKIAATAANAAPANDKFTVFEGAFNKAIKESTGGAYESYKFIPTLEAAVKQAYAATVAAAP
Poap5      AATSDATYKAAVGATPEAKFDSFVAAFTEALRIIAGVLKVHAVKPITEETGAAKIPAGEQQIIDKIDAAFKVAATAANAAPANDKFTVFEAAFNNAIKESTGGAYDTYKSIPSLEAAVKQAYAATIAAAP
Lolp5b     DAALKLAYEAAQGATPEAKYDAYVATLTEALRVIAGTLEVHAVKPAAEEVKVGAIPAAEVQLIDKVDAAYRTAATAANAAPANDKFTVFENTFNNAIKVSLGAAYDSYKFIPTLVAAVKQAYAAKQATAP
Consensus  dAa...aYkaA.GaTPEAK%Da%YA.lTEALR!IAG.LeVHAVKPaaEEv..akIPAgE.Qi!DK!DAA%k.AATAAnaAPA#DKFTVFE.aFNnAIKeStGgAY#sYKfIPtLeAAVKQAYAAt.AaAP

           261     270       280       290       300       310       320       330       339
           |--------+---------+---------+---------+---------+---------+---------+---------|
Phlp5      QVKYAVFEAALTKAITAMSEVQKVSQPATGAAT-------VAAG-------------AATTATGAASGAATVAAGGYKV
Dacg       EVKYAVFEAALTKAITAMSEAQKVATPAA-----------VATG-------------AATAAASAATGAATAAAGGYKV
Poap5      EVKFAVFKAALTKAITAMAEVQKVSKPVAGAATVAAGAATAATG-------------AATGAAGAATGAATVSAGGYKV
Lolp5b     EVKYTVSETALKKAVTAMSEAEKEATPAAAATATPTPAAATATATPAAAYATATPAAATATATPAAATATPAAAGGYKV
Consensus  #VK%aVfeaALtKA!TAMsEa#KvatPaa.a......aa..Atg.............AattAt.AA.gAataaAGGYKV
```

Grass pollen allergens

How to diagnose the right grass pollen allergy ?

Poa pratensis, Festuca rubra, Phleum pratense,
Dactylis glomerata, Arrhenaterum elatius, Lolium perenne,
Holcus lanatus, Agrostis stolonifera, Anthoxanthum odoratum,
Alopecuris pratensis, Secale cereale, Triticum vulgare,
Hordeum vulgare, Avena sativa, Zea mays,
Phragmites communis, and *Cynodon dactylon*.

One grass species is sufficient for in vitro diagnosis of grass pollen allergy. With purified natural Lol p 1and Lol p 5, greater than 90% of grass-positive sera is detected.

Around 80% of the IgE response to grass pollen is directed to these major allergens.

van Ree et al. 1998
JACI 102

Grass pollen allergens

Phl p 1 = Dac g 1 = Poa p 1 = Lol p 1

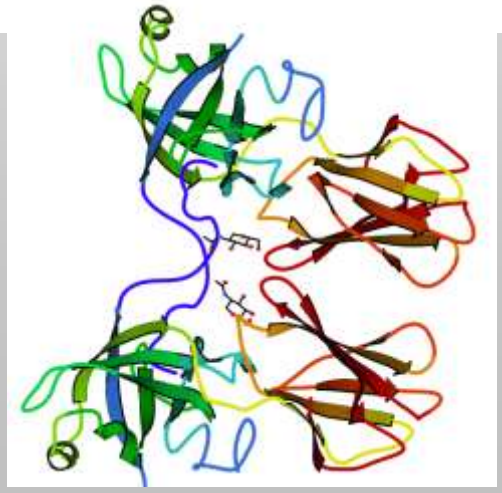
always ?

Phl p 1 versus Bet v 1

```
           1       10        20        30        40        50        60        70        80        90       100       110       120       130
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Phlp1      MASSSSVLLVVVLFAVFLGSAYGIPKVPPGPNITATYGDKWLDAKSTWYGKPTGAGPKDNGGACGYKDVDKPPFSGMTGCGNTPIFKSGRGCGSCFEIKCTKPEACSGEPVVVHITDDNEEPIAPYHFDL
BETV1A                                                                                                                    MGVFNYETETTSVIPAARLFKA
Consensus  ..........................................................................................................................egVfnheT#dneeiiAarhFda

           131     140       150       160       170       180       190       200       210       220       230       240       250       260
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Phlp1      SGHAFGAMAKKGDEQKLRSAGELELQFRR---VKCKYPEGTKVTFHVEKGSNPNYLALLVKYVNGDGDVVAVDIKEKGKDKWIELKESWGAIWRIDTPDKLTGPFTVRYTTEGGTKTEAEDVIPEGWKAD
BETV1A     FILDGDNLFPKVAPQAISSVENIEGNGGPGTIKKISFPEGFPFKYVKDRVDEVDHTNFKYNYSVIEGGPIGDTLEKISNEIKIVATPDGGSILKISNKYHTKGDHEVKAEQVKASKEMGETLLRAVESYL
Consensus  fghagda$akKgaeQairSae#iEg#grr...kKck%PEGfkfk%hk#rgd#p#hlallynYsng#Gdp!addieeign#ikIeakedgGaIlrIdnkdhlkGdheVraeqegasKeeaEdliragekad

           261  268
           |------|
Phlp1      TSYESK
BETV1A     LAHSDAYN
Consensus  laheda..
```



Overview

Allergen	Biochemical name	MW (SDS_PAGE)
Art v 1	Defensin + Prolin-rich	28 (theor: 10.8)
Art v 2		20 (35 non-red)
Art v 3	Lipid transfer protein	12
Art v 4	Profilin	14
Art v 5	Polcalcin	10
Art v 6	Pectate lyase	44
Amb a 1	Pectate lyase	38
Amb a 2	Pectate lyase	38
Amb a 3	Plastocyanine	11
Amb a 5		5
Amb a 6	Lipid transfer protein (LTP)	10
Amb a 7		12
Amb a 8	Profilin	14
Amb a 9	Polcalcin	10
Amb a 10	Polcalcin	18

Biology of allergens

More and more allergen sequences known
Distribution into protein folding families limited

Chapman et al. (2007) JACI 119

Allergens found in 29 from 2615 families

Radauer & Breiteneder (2006) JACI 117

The big players:

Pathogenesis-related group 10 proteins (Bet v 1)

Expansins (grass group I)

Profilins

Calcium-binding proteins / polcalcins / EF-hand proteins (Ole e 3, Phl p 7)

Lipid-transfer-proteins (LTPs) (Pru p 3)

Peaches an Cherries

Sensitization to Prunus fruits in Central and Northern Europe

mainly due to PR10 proteins

→ Cross-reaction of birch-pollen allergic patients

Sensitization to Prunus fruits in Southern Europe

mainly against non-specific lipid-transfer protein (nsLTP)

clinically severe cross-reactions

Ballmer-Weber (2002) Allergy 57

Biology of allergens

Others:

Defensin-like (Art v 1)

Ribonucleases (grass group V)

Cupin-like / vicillins (Ara h 1)

PR5-proteins / Thaumatins

Pectate lyases

Ole e 1-like (unknown function)

Proteases (Actinidin in kiwi)

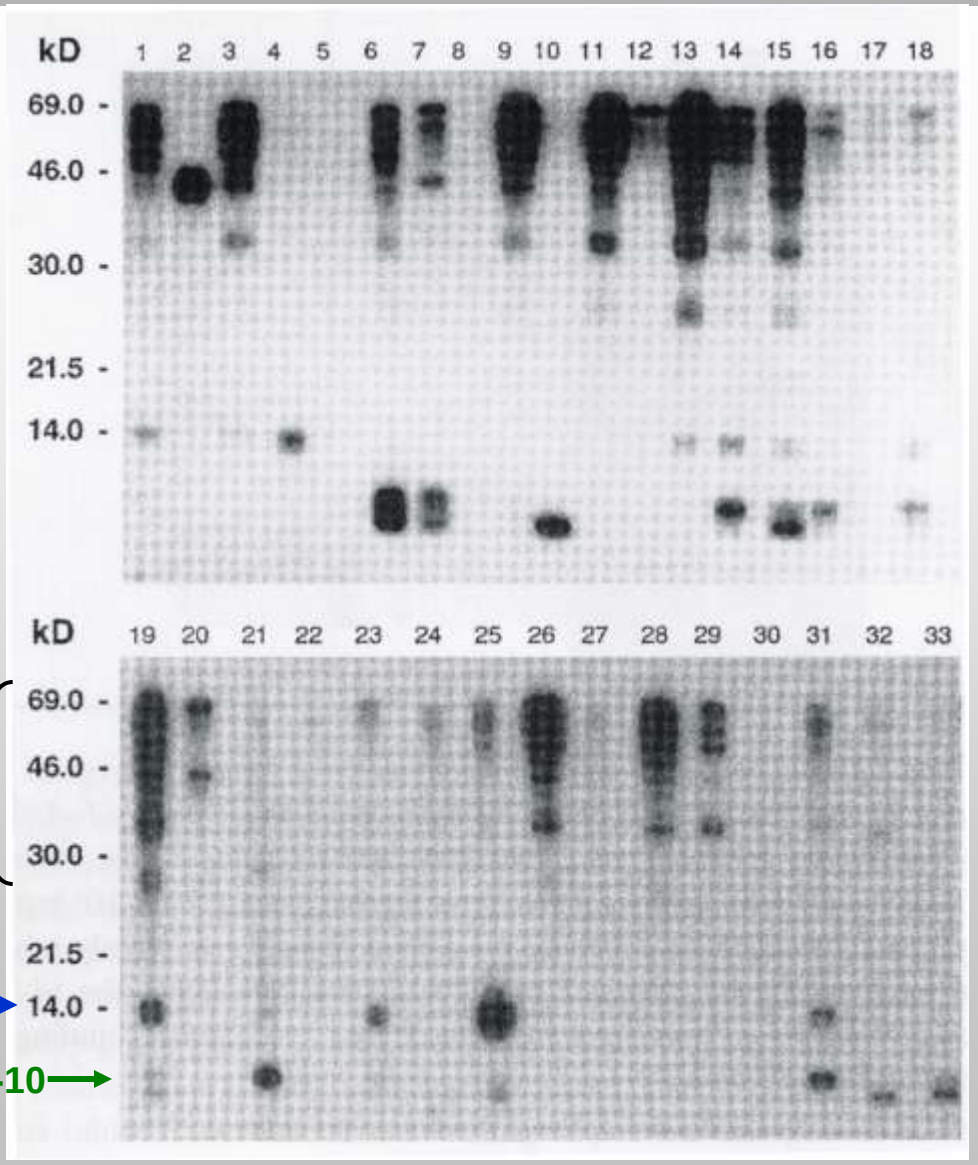
….

Low vs. High MW allergens

IgE-immunoblot against extracts of oil seed rape pollen

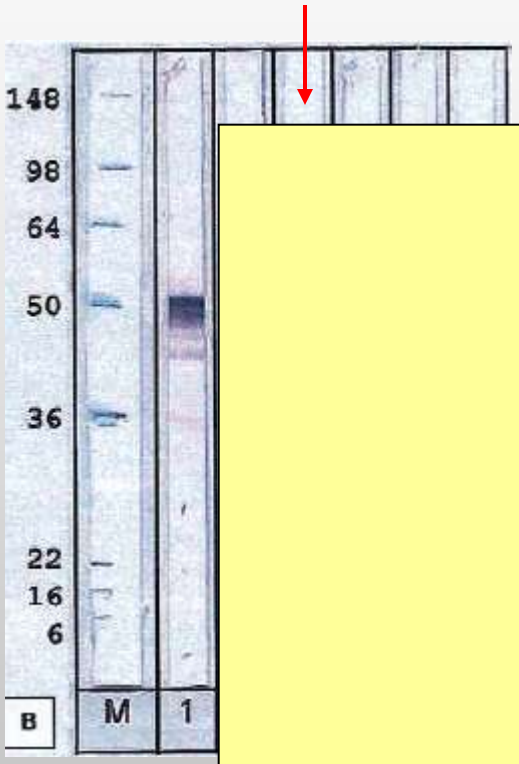
from

Focke *et al.* (1998) IAAI 117



Inhibition with glyco-conjugates

IgE binding to tomato extract



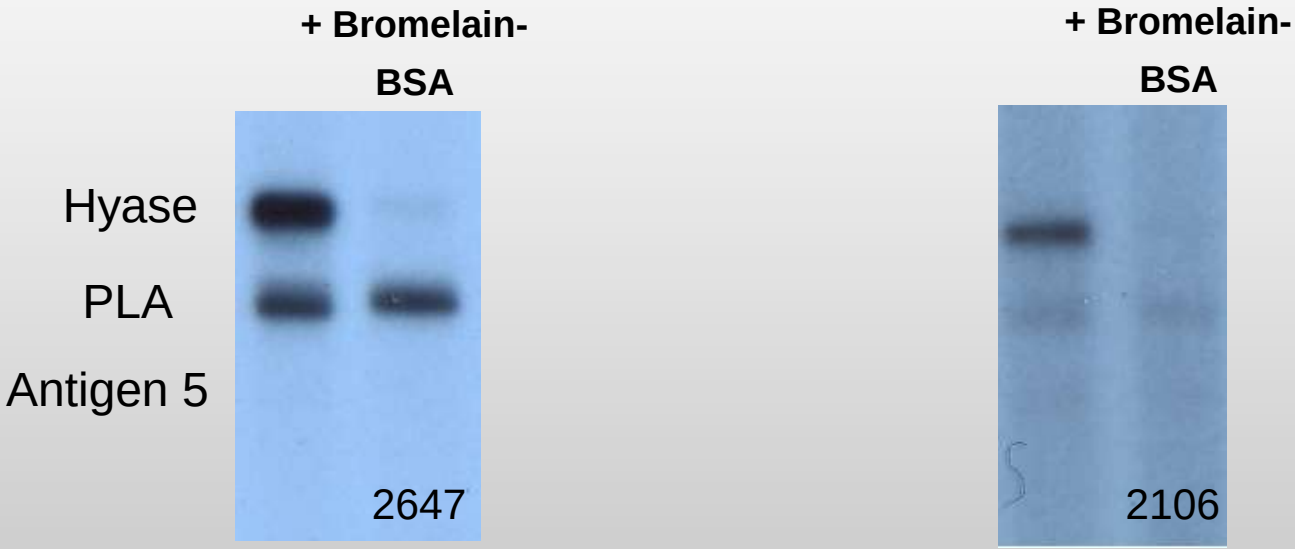
lane 1, serum pool (n = 3)

Foetisch *et al.* 2003, JACI 111

Inhibition with glyco-conjugates

IgE binding to yellow jacket venom

Hyaluronidase is the only glycoprotein in V. vulgaris venom



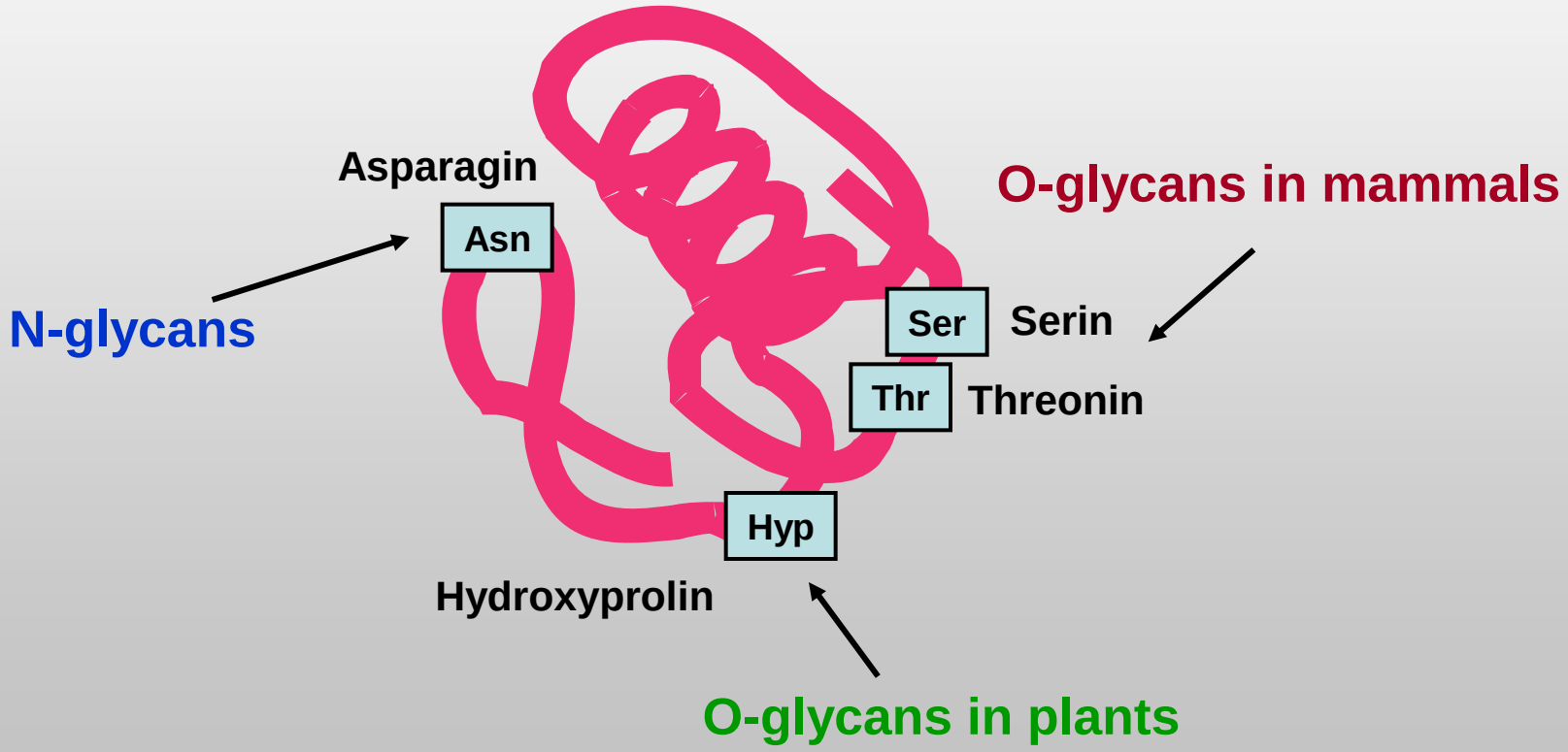
Only reaction to PLA based on protein

Only CCD reactivity.

Jin, Hemmer, Focke, Altmann, unpublished

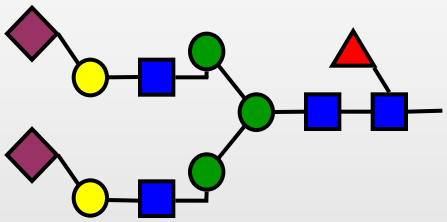
Glycoproteins

In glycoproteins, oligosaccharides are covalently attached to the polypeptide, either via NH_2 groups or via OH groups



Glycoproteins

A mammalian N-glycan



Neu5Acα–6Galβ–4GlcNAcβ–2Manβ

Fucα

6

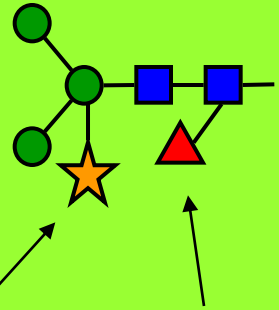
6

3Manβ–4GlcNAcβ–4GlcNAcβ–Asn

Neu5Acα–6Galβ–4GlcNAcβ–2Manβ

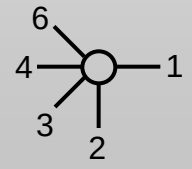
High diversity of structural elements

A plant N-glycan



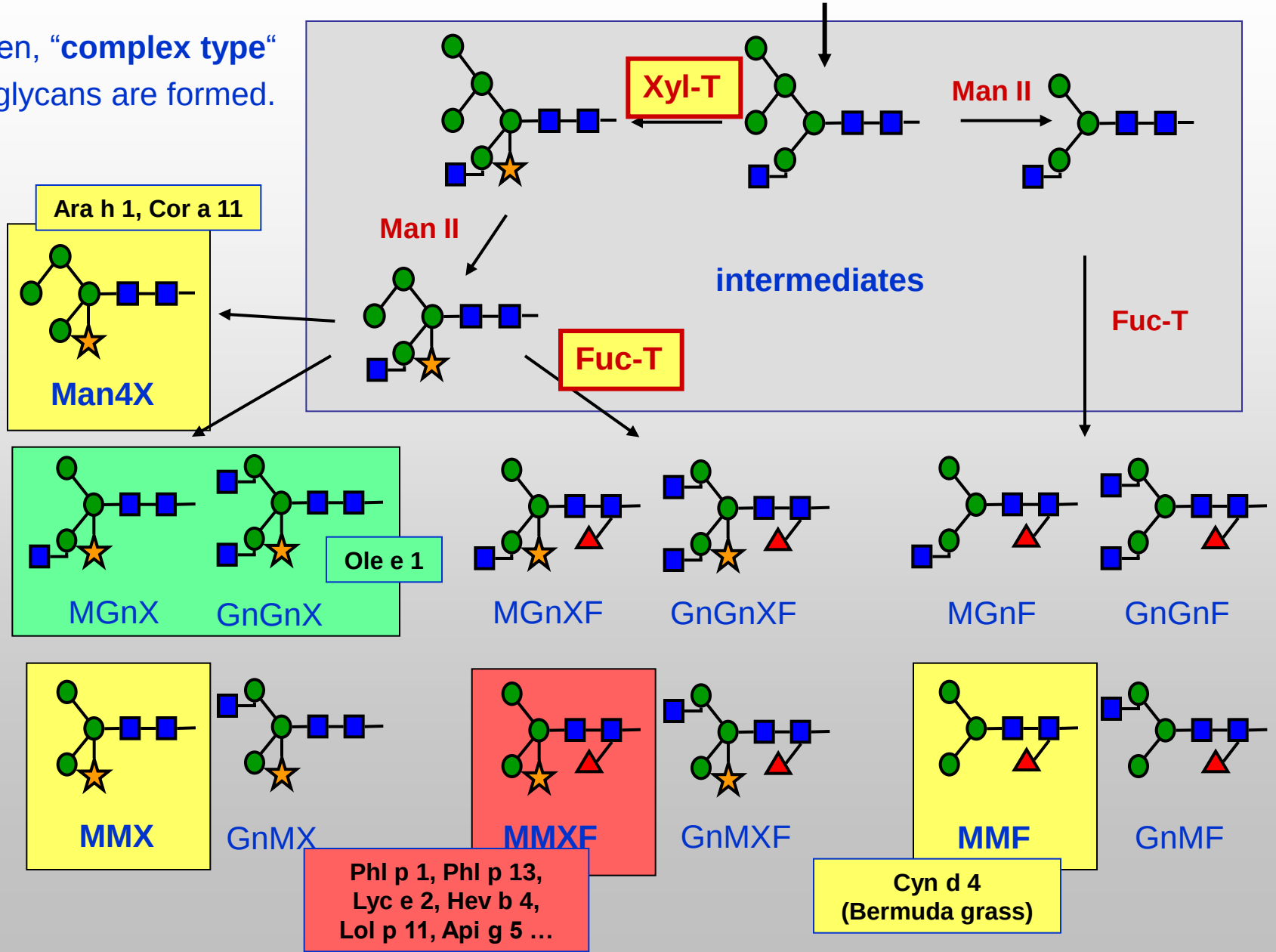
Xylose and core 3-fucose are foreign to mammals = immunogenic

GlcNAc	*N*-acetylglucosamin
Gal	galactose
Fuc	fucose
Xyl	xylose
Neu5Ac	*N*-acetylneuraminic acid

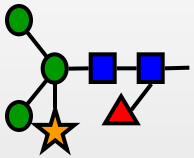


Plant N-glycans

Then, "**complex type**"
N-glycans are formed.



Plant N-glycans



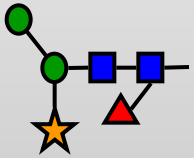
MMXF

usually major component of plant glycoproteins

e.g. allergens **(Phl p 1, Phl p 2, Lol p 11, Api g 5, Hev b 4, Lyc e 2 etc.)**

lectins (*Sophora japonica*, *Erythrina* spp. etc.)

enzymes (horseradish peroxidase, ascorbic oxidase etc.)

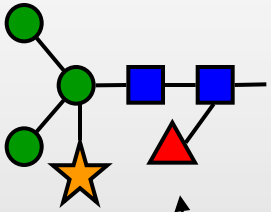


MUXF

rare, *e.g.* bromelain

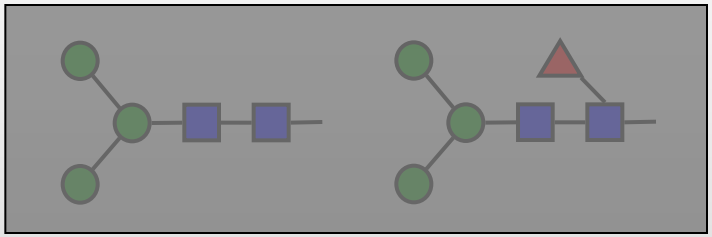
Insect N-glycans

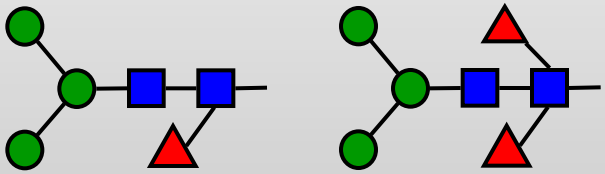
A plant N-glycan



Xylose and core 3-fucose are foreign to mammals = immunogenic

Insect N-glycans





Core 3-fucose in insect venoms and in neuronal tissues

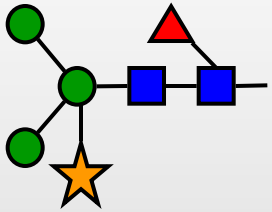
Api m 1, Ves v 2 …

Altmann *et al*. 1999, Glycoconjugate J. 16

"cross-reactive carbohydrate determinants" (CCD)

N-glycans from molluscs and parasites

A mollusc N-glycan

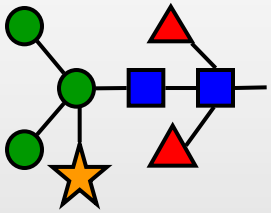


Xylose as in plants
core α1,6-fucose as in mammals

but also many larger structures

in snails and mollusces
e.g. on hemocyanin from *Helix pomatia*

N-glycan from a parasitic helminth



Difucosylation and xylose

also many larger structures which contribute to immunogenicity

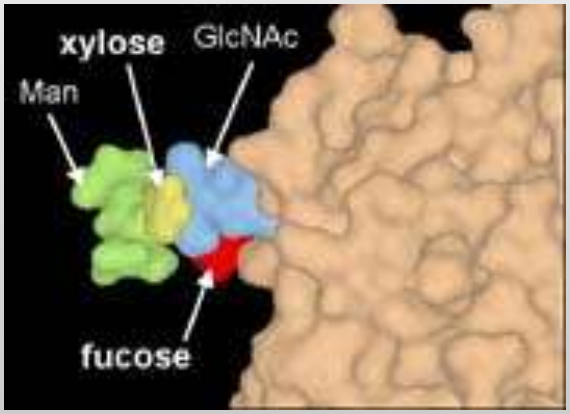
in *Schistosoma* or *Haemonchus* species

Hokke *et al.* 2005, Parasitol. Immunol. 27

Van Die. 1999, FEBS Lett. 463

IgE against glycans

The role of xylose and core-α1,3-fucose in allergy



from www.faz.at

IgE against glycans

CCDs are:

View 1: **peptide epitopes**
glycan just confers conformational difference

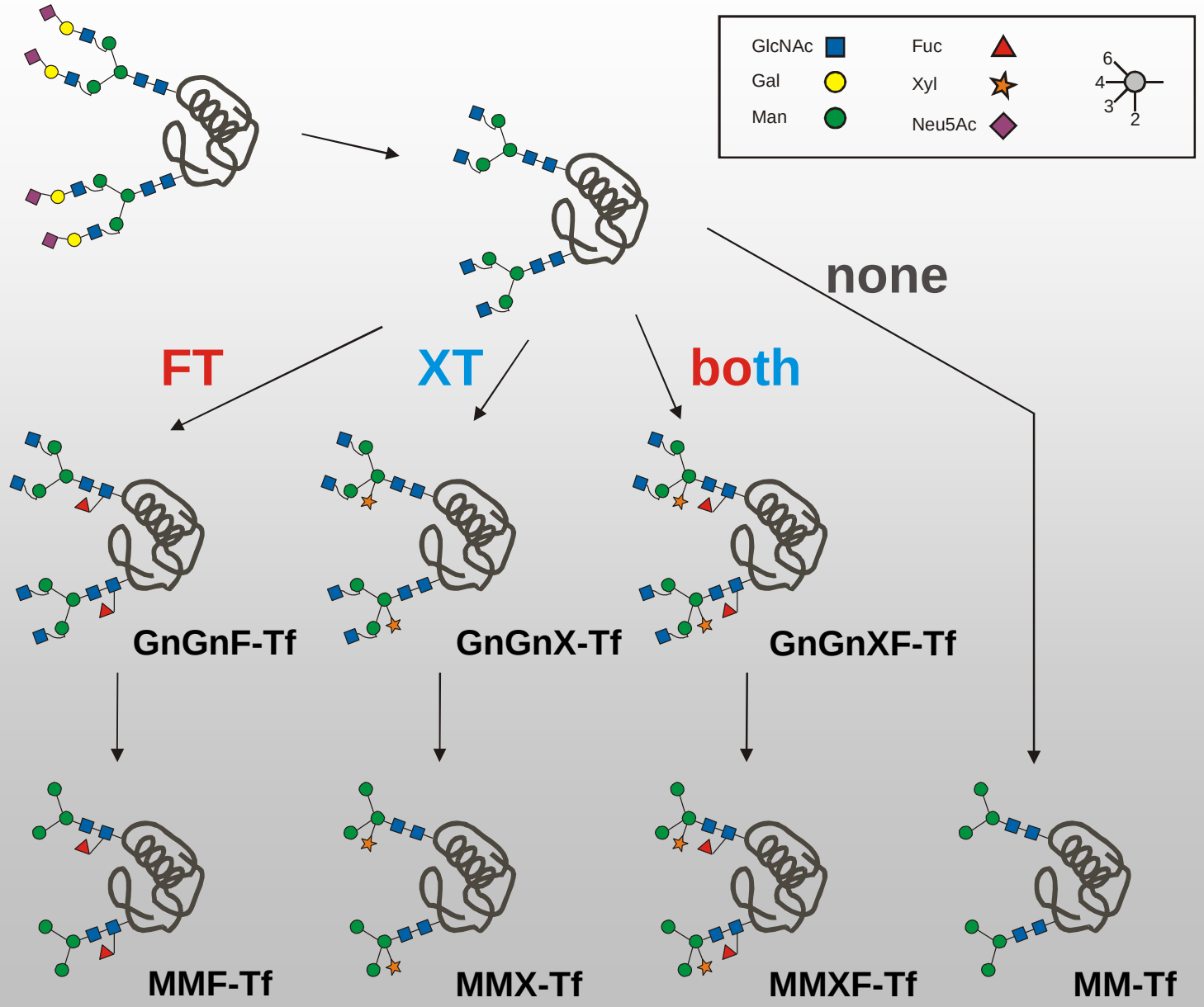
View 2: **carbohydrate epitopes**

older proofs: periodate oxidation of sugars

modern: specific inhibition

most modern: generation of epitope with recombinant enzymes

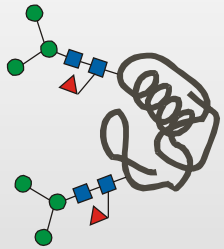
Biosynthetic glyco-allergens



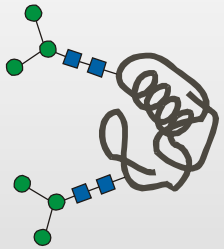
IgE binding to glyco-allergens

Coat-allergens

artificial CCD-allergen (MMF-Tf)



control (MM-Tf)

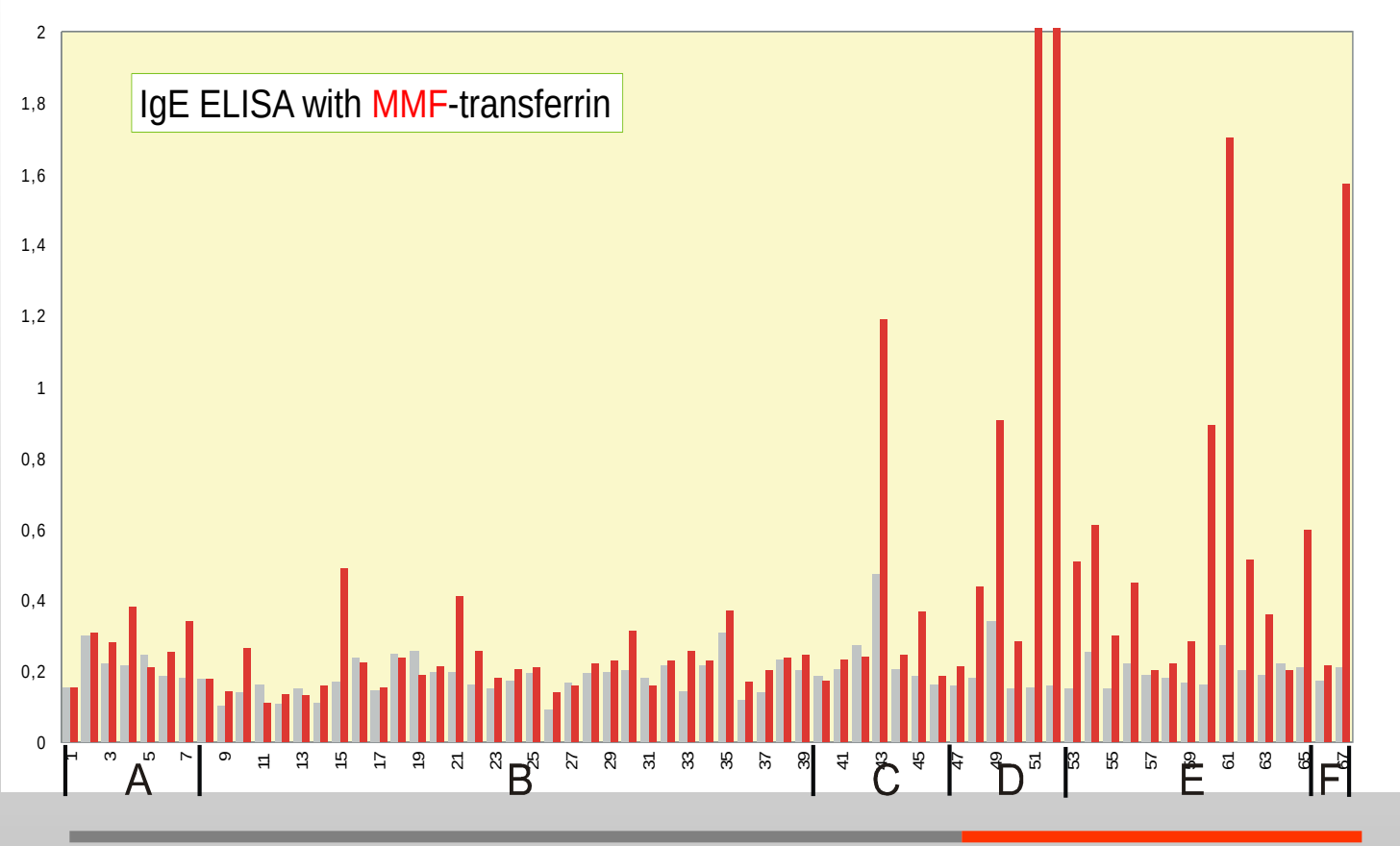


Patients´ sera

Expected to be CCD-negative:
with narrow sensitization

Expected to be CCD-positive:
bee/wasp venom, oil seed rape pollen, polyvalent

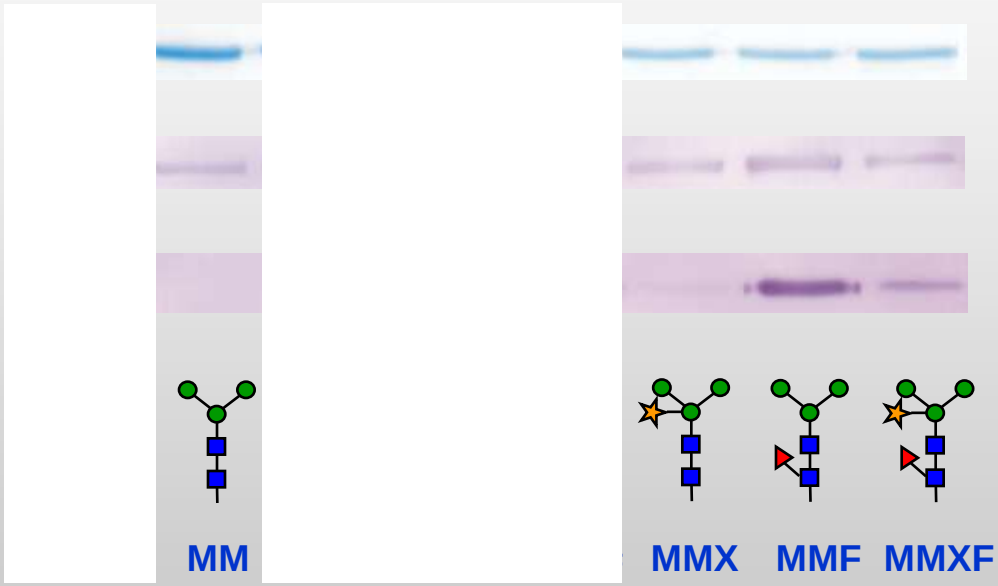
IgE binding to glyco-allergens



Allergy of patients, whose sera were used for ELISA: A-birch pollen, B-grass pollen, C-ash, D-bee/wasp venom, E- oil seed rape pollen, F-polyvalent

Bencurova *et al*. 2004 Glycobiology

Immunoblot of glycomodified transferrins with human CCD-reactive serum (pool of 19)



Conclusion: h-IgE binds to core α1,3-fucosylated N-glycans

h-IgG is less specific (strong binding only to fucosylated glycans)

IgG characterization

Specificity of IgG / IgE

Rabbit IgG specifically recognizes two different epitopes on N-glycans: one with Fuc – one with Xyl

Mouse (or rat) IgG lacks this specificity

Human Abs more like rabbits

Summary of first part I

20 % (+/- 5) **of allergic patients have anti-CCD IgE**

Asn-linked glycans with a1,3-fucose are the CCD epitope

Such glycans are found on allergens (and pseudoallergens) from pollens, vegetable food and insect venoms.

Summary of first part II

IgE-binding to CCD epitopes is an important source of false-positive RAST diagnosis. It can be specifically inhibited.

because

Clinical symptoms due to CCD-epitopes have not been proven so far and are certainly the exception

The probably most important reason for irrelevant *in vitro* results are protein-linked carbohydrates

Cross-reactive carbohydrate determinants "CCDs"

Aalberse *et al.* 1981, JACI 68

Clinical relevance of CCDs

All epitopes are equal. Are some less equal ?

valency

glycoproteins incapable of crosslinking IgE ?

many glyco-allergens monovalent

(Phl p 1, Ara h 1, Api m 1, bromelain etc.)

but many polyvalent (Lyc e 2, Ves x 2, **HRP** etc.)

affinity

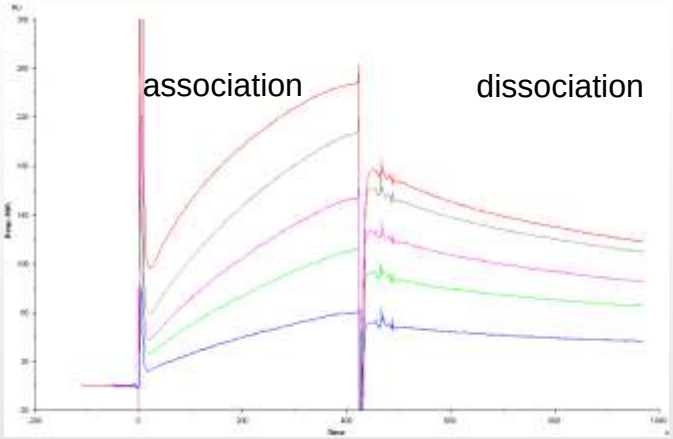
low affinity ?

but:

histamine release data

affinity of human IgG and IgE

Affinity of human anti-CCD IgE



[IgE]

0.06 -

0.6 nM

Affinity determination by surface plasmon resonance (SPR)

Jin, Hantusch, Hemmer, Altmann, unpublished

Glycoform on sensor chip	Antibody class	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
MMF-transferrin	IgE	7.8×10^5	4.9×10^{-4}	**0.63**
	IgG	6.5×10^3	1.4×10^{-4}	**20.7**
MMXF-transferrin	IgE	1.1×10^7	7.4×10^{-4}	**0.068**
	IgG	1.1×10^3	1.3×10^{-4}	**11.1**

Low affinity cannot explain the benign conductance of CCDs !

Affinity of human anti-CCD IgE

Comparison of human anti-CCD IgE and IgG by ELISA

	Glycan epitopes	Phl p 5a	Bet v 1
	K_D (M)		
IgE	**0.68 – 6.3 x 10^{-10}**	**2.8 x 10^{-10}**	**0.22 x 10^{-10}**
IgG	**1 – 2 x 10^{-8}**	**180 x 10^{-8}**	**49 x 10^{-8}**
ratio	**32 / 163***	**6430**	**2227**

* MMF / MMXF

Hantusch et al. 2005, Immunol. Lett. 97

Why this difference ?

Daily confrontation with CCD-containing materials acts like sublingual immune therapy (“allergy vaccination”)

Glyco-SLIT

Allergens with O-glycans

Number of allergens currently known to contain IgE-binding O-linked carbohydrate

1

An O-glycosylated plant allergen

Art v 1 is the major allergen from mugwort pollen

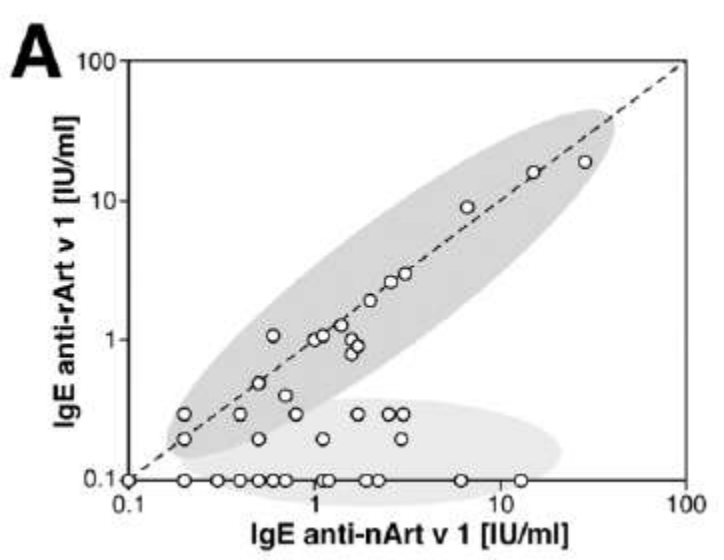
**Art v 1 consists of a globular defensin-like domain and
a proline-rich, partially hydroxylated “tail“**

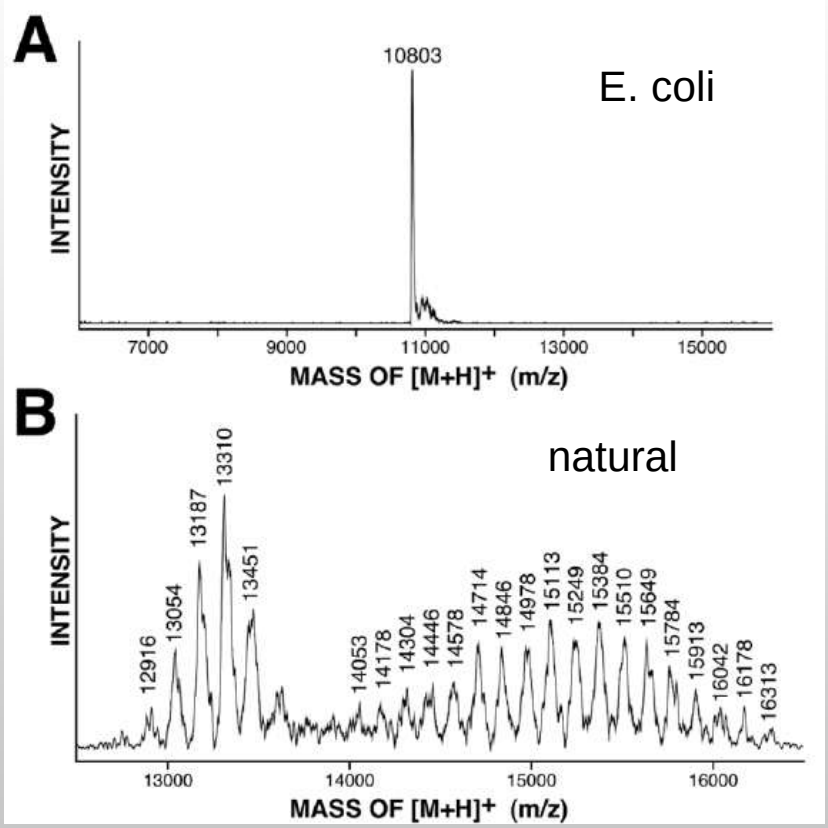
**It contains Gal and
Ara in a molar ratio
of 1 : 11.4**

Model of Art v 1 protein backbone

Mugwort pollen allergen Art v 1

An O-glycosylated plant allergen

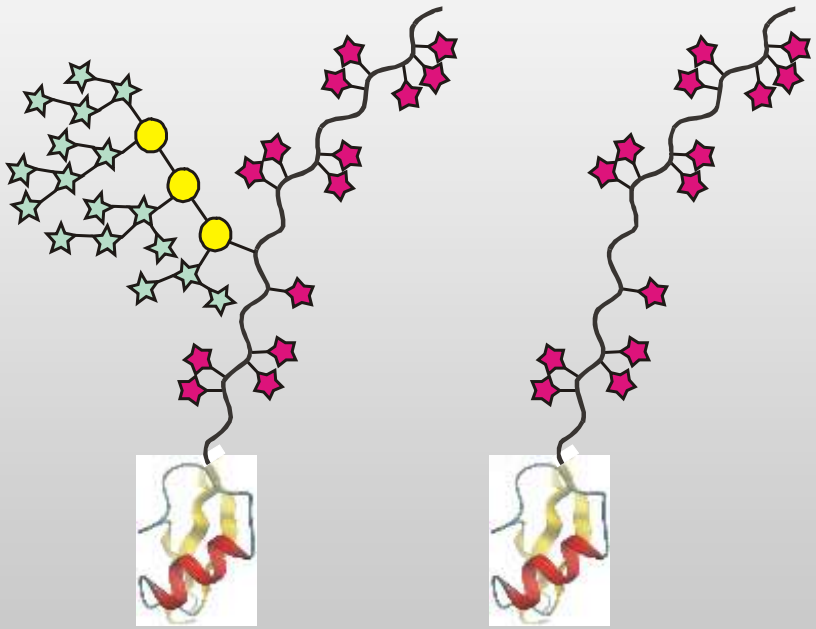




Sera of mugwort sensitized patients react different with recombinant and with natural Art v 1.

Model of the glycoprotein Art v 1

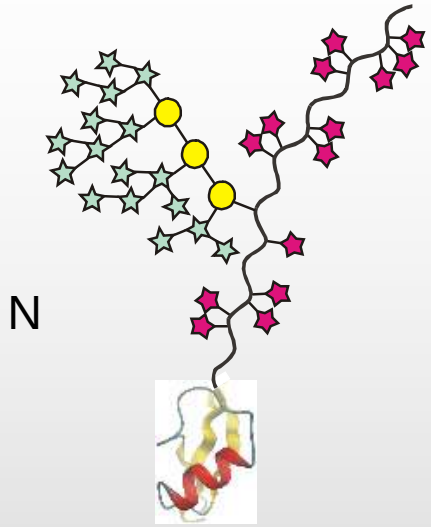
The mugwort allergen Art v 1 occurs with and without the polysaccharide

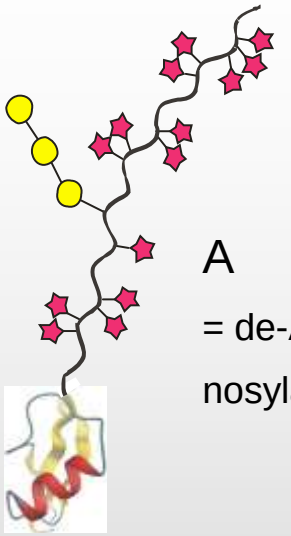


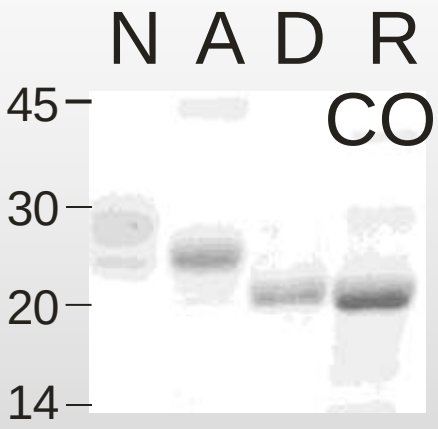
N … natural Art v 1

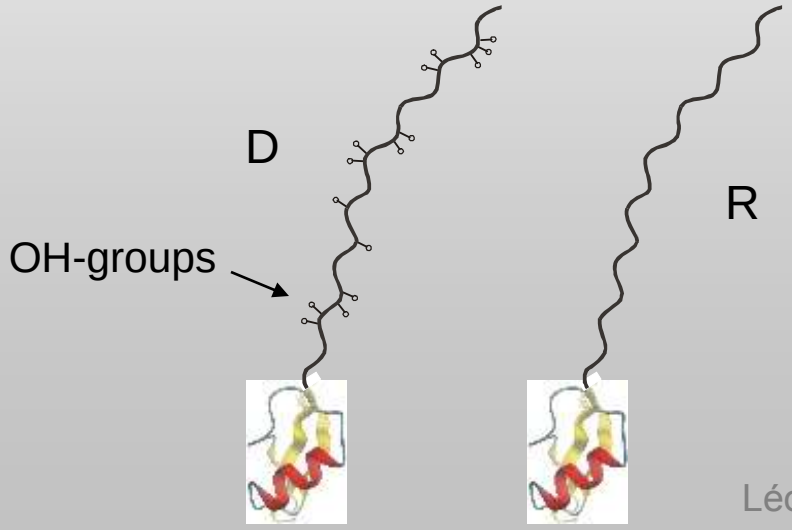
Léonard et al. 2005
JBC 281

IgE binding by Art v 1 sugars









N … natural Art v 1

A … without arabinan

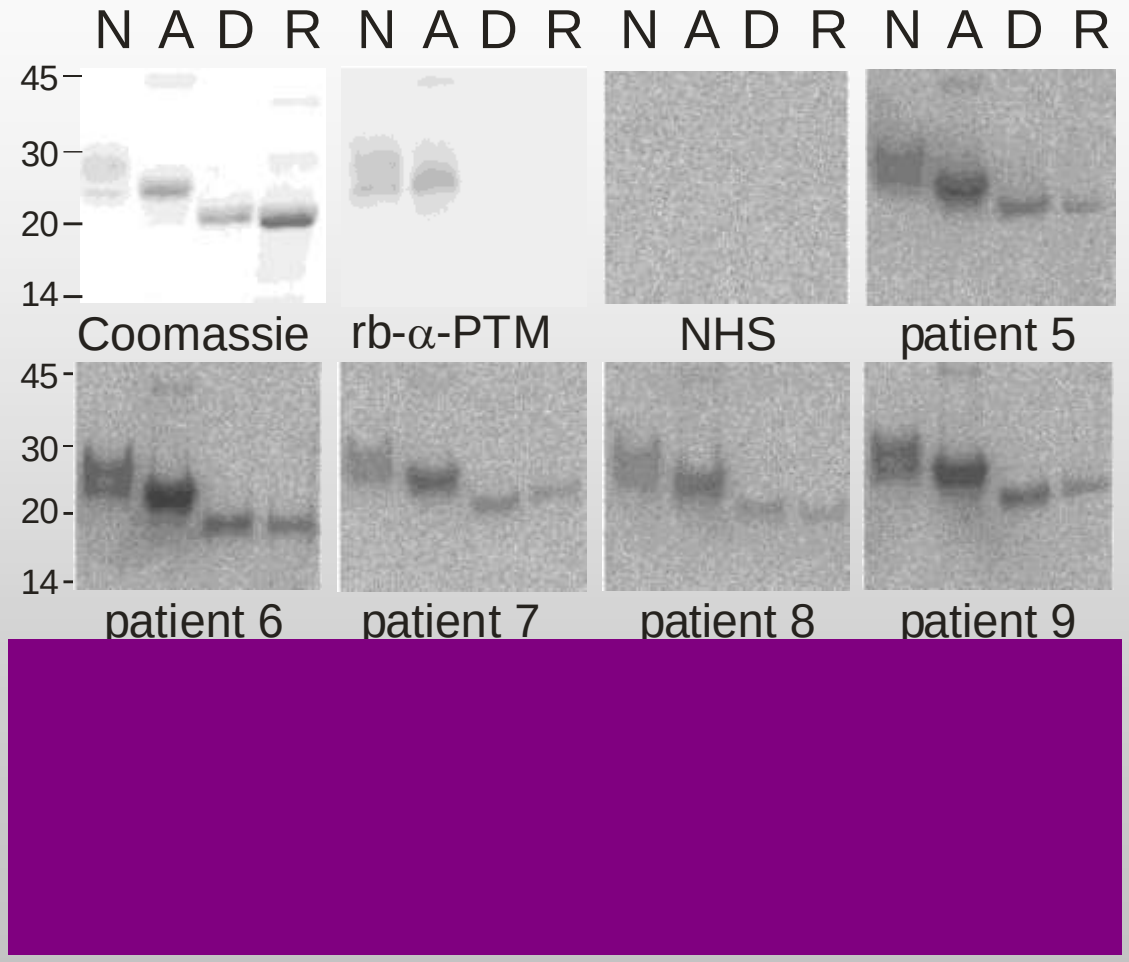
D … deglycosylated

R … recombinant

Léonard et al. 2005
JBC 281

IgE binding to Art v 1 glycoforms

Some patients do not bind to deglycosylated Art v 1



N … natural Art v 1

A … without arabinan

D … deglycosylated

R … recombinant

IgE binding to Art v 1

Art v 1 carries two types of novel hydroxyproline-linked carbohydrates

The single β-arabinofuranoses are responsible for binding of rabbit IgG and human IgE

Similar glycoproteins have not yet been identified

Acknowledgement

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Martin Himly
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Other institutions

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Stefan Vieths
Arnd Petersen

… and all those who have provided wonderful pictures on the internet