Reconciling Actual Risks of Food Allergy with Scientific Risk Assessment, Regulatory and Public Demands

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ProImmune – Immunogenicity

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UNIVERSITY OF NEBRASKA-LINCOLN

AllergenOnline: Home of the farrp allergen protein database

Food/Feed Safety Assessment
RELATIVE SAFETY

Historically: we learned to eat "safely" through experience but some "safe" foods cause disease in some people:

- Wheat must be avoided by those with celiac disease
- Most legumes (beans/peas) must be cooked to inactivate lectins and trypsin inhibitors or diarrhea and malnutrition
- Allergic individuals must avoid specific foods causing their allergy

We can markedly reduce big-risks from new sources (Genetically Modified Organisms, novel food Ingredients), but we can not yet guarantee lack of immunogenicity, allergy or absolute safety!

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Normal Immune Response to Dietary Proteins

- Tolerance
- Ignorance
- True for most proteins, most foods, most people
- Allergic reactions and celiac, relatively rare compared to influenza…etc.
- Great range of responses to dietary proteins….all allergic responses are not equal, allergens are not all equal

Food Allergy – Growing Concerns / Perceived Risks from different perspectives

- **Consumers**
  - Apparent increasing prevalence / diagnosis of food allergy
  - Few individuals with severe – life threatening, life-long risks
  - Complex foods, multiple sources
  - Unclear labeling, news reports & social media reports of risks
- **Food Producers**
  - Liability, regulations, recalls
  - Labeling, sourcing, value added product competition
  - International trade, languages, different regulations
- **Regulators**
  - Consumer pressure
  - Uncertain scientific methods for risk evaluation
  - Complex detection issues
  - International trade – complexity of regulation and foods
  - Large / small producers, restaurants, packaged foods

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Are There Cures for Celiac or Food Allergy?

- Not in the near future……therefore:
- AVOID YOUR ALLERGEN or Gluten…IF you are sensitive
- Allergenic foods and gluten must be labeled accurately to protect those with allergies or celiac disease !

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Types of Foods / Sources and Processes Potentially Requiring Regulatory Assessment: USA, EU, Japan…

- Genetically Modified Organisms (plants, animals, fungi, prokaryotes)
- Cloned animals
- Purified ingredients from allergenic or sometimes highly divergent sources (e.g. any legume)
  - Proteins (or ingredients with potential protein contaminants such as flavor ingredients, gums, new sweeteners, oils, starch, mucins)
- Processed “hypoallergenic” foods
- Goal: Protect specifically allergic consumers
- Intent to predict “new allergens”, new sensitization?
- CAUTION: Perform “scientifically interesting” tests at your own peril. Those results can impact possible approvals!

GMOs…detailed food allergenicity assessment

- FDA wants early consultation, tests on case-by-case
- EPA more stringent and less communicative
- Many countries want full submission of proscribed studies, no discussion
- EFSA (European Food Safety Authority) keeps pushing toward animal model tests for some GMO, and evaluate potential changes in endogenous allergen expression, transcriptomics etc. They have suggested adding immunogenicity and immunotoxicology…. AND POST-MARKET SURVEILLANCE

Novel Food Ingredients…detailed allergenicity assessment requirements less clear

- FDA wants developers to have early conference to agree to study process on case-by-case: GRAS or New Food Ingredient
- EU and some other countries have specific requirements, but not procedures
- There has been less effort to develop consistent guidelines, primarily focused on avoiding risk of exposure to major allergenic proteins
- PRECAUTIONARY ENVIRONMENT is becoming common

Food Safety Evaluations:
We need to differentiate…

- Scientifically justified risks / concerns
- Scientifically justified risk assessment
- Scientifically justified control measures

From

- Basic “interesting” scientific questions without direct relevance to safety (e.g. unintended effects)
- Irrational fears and unsubstantiated speculation
- Non-predictive tests
- Impractical and overly restrictive controls

Immunological Adverse Food Reactions

IgE-Mediated allergy

- Systemic (Anaphylaxis)
- Oral Allergy Syndrome
- Immediate gastrointestinal allergy
- Asthma/rhinitis
- Urticaria
- Morbilliform rashes and flushing
- Contact urticaria

Non-IgE Mediated Cell-Mediated

- Eosinophilic esophagitis
- Eosinophilic gastritis
- Eosinophilic gastroenteritis
- Atopic dermatitis

- **Celiac Sprue**
- Protein-Induced Enterocolitis
- Other Protein-Induced Enteropathy
- Eosinophilic proctitis
- Dermatitis herpetiformis
- Contact dermatitis

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Prevalence and Severity

- Celiac Disease affects nearly 1.3% of the world population
  - Genetically linked MHCII DQ2 and DQ8 (but >20% of all people are DQ2 or DQ8, small # have disease)
  - Small percent with celiac must avoid even 100 mg of wheat, barley or rye grain
- Food Allergy 3-6% of world population
  - >20 genes, none are dominant
  - Hundreds of foods, few proteins (out of hundreds) in each food are allergens ALLERGENS are NOT all EQUAL!
  - Few allergenic foods cause life-threatening allergies
  - 0.8% in USA allergic to peanut, 1% to 5% of those have severe allergies and are at risk of systemic anaphylaxis …rice, beef, chicken and bacteria rarely cause food allergy

Celiac Disease with wheat gluten: variable exposure effects

Almost normal intestine, but eosinophils in tips of villi, mild inflammation

Severe celiac inflammation, flattened villi, malabsorption, wasting disease, autoimmune

T-cell and IgG against modified peptides (self-transglutaminase) and natural peptides, and finally modified human connective tissue---autoimmune disease

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IgE Mediated Food Allergy Sources

Diagnosis of IgE Mediated Food Allergies

In Vivo

- Clinical history
- Elimination diet
- Skin Prick test (SPT) with extracts or prick-to-prick
- Food challenge
  - Placebo and suspected food

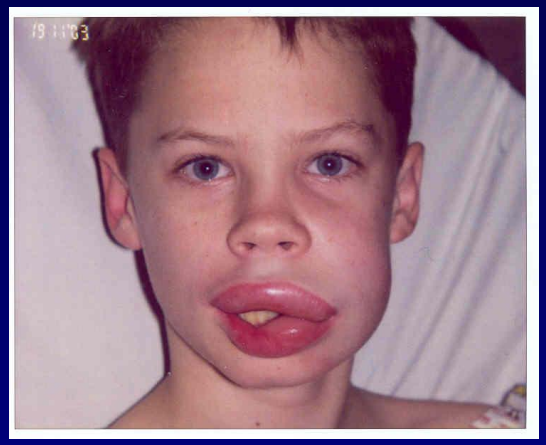
In Vivo (challenge) Limitations

- Some risk to patient
- Time consuming (full- day)
- Qualitative
- Only test one or two foods

In Vitro (IgE)

- Specific IgE measurement
  - CAPS (Pharmacia Diagnostics)
  - RESEARCH METHODS
  - ELISA/RAST
  - Western blots
- Positive result suggest, DO NOT prove allergenicity

What is IgE mediated food allergy – symptoms - pathology



Food allergy causes more than just a runny nose or urticaria !

Sometimes mixed IgE, T-cell and eosinophil reactions

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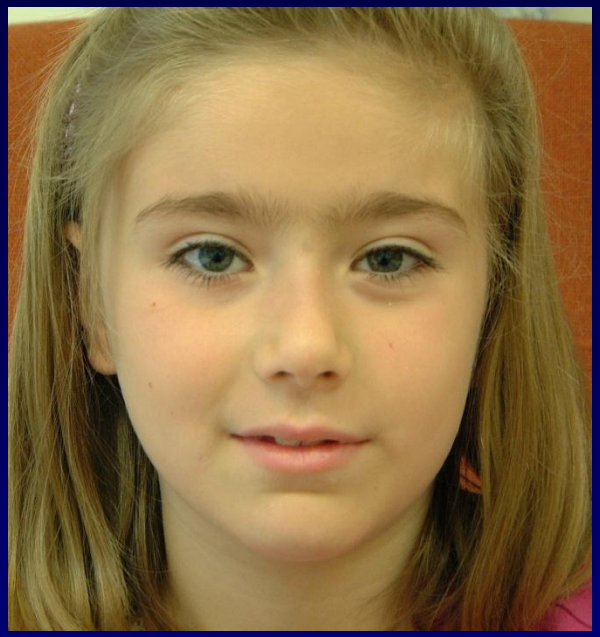

The unexpected…
15 minutes

During a food challenge.
Some risk

But she did recover:
Epinephrine, IV
Other support
Clear anaphylaxis

Could have died if this was not in the clinic

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Food Allergy Prevalence (apparently increasing estimates from US population of 300 million)

- ~ 30% of people have allergies to inhaled allergens
- IgE mediated allergies (Type I) is the most common type
- Occurrence of food allergy in the US and Europe
  - 2-4% of adults
  - 4-8% of young children
  - Severe reactions relatively rare (U.S. *estimates:* ~100,000 Emergency Room visits, < 200 fatal reactions /year)
- Eight foods account for ~ 90% of food allergies & even minor ingredients require labels (US), 14 EU…some countries do not:

Peanuts	Milk	(Wheat?)
Eggs	Fish	(Soybeans?)
Crustacea	Tree nuts	

The EU adds lupine, celery root; mustard and sesame seeds

India should add chickpea, blackgram, lentil, pigeon pea?

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Known Allergenic Proteins

Very few foods or proteins represent major risks

- Peanuts
  - Probably ~ 50 to 80+ deaths per year in the U.S.
  - 3 to 5 major allergens, 5 to 7 minor allergens
  - 10,000-40,000 total genes
- Soybeans
  - < 1 fatal reaction per year in the U.S.
  - 3 to 5 moderate allergens
  - ~20,000 total genes
- Cow's milk
  - Few published reports of fatal reactions (e.g. Macdougall, 2002)
  - Caseins and beta-lactoglobulin dominant allergens, also alpha lactalbumin, minor allergens IgG, serum albumin
- Fish
  - Few reports of fatal reactions, but strong reactions common
  - 1 major allergen (parvalbumin), 2 to 4 minor allergens

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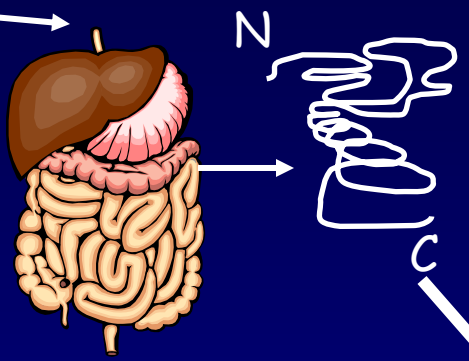
IgE – allergy: Sensitization vs. Tolerance



- Protein digestion
- Antigen (Ag) absorption
- Ag processing in DC, MΦ, B cells
- Ag presentation to T cells
- T cell and B cell memory

APC

·IFNγ
·TNF-α
·IL-5

T c cell

EOS

·IL-4
·IL-13

B cell

T h cell

·IL-2
or
·TGFb

T reg cell
?
Tolerance

Non-IgE-Mediated

Allergy
Mast cell

IgE-receptor

IgE

IgG
or
IgA

Histamine

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Elicitation: Protein-specific IgE is the key mediator of specificity in Food Allergy

IgE Mediated Symptoms

10 to 20 minutes after eating:

- hives
- angioedema
- asthma
- diarrhea/vomiting
- atopic dermatitis
- anaphylaxis

Sensitization
Antigen
Specific
B cells
Make IgE

Peanuts

(Ara h 1)

(2 IgE epitopes)

IgE

FcεRI

Mast cells
release
histamine
& leukotrienes

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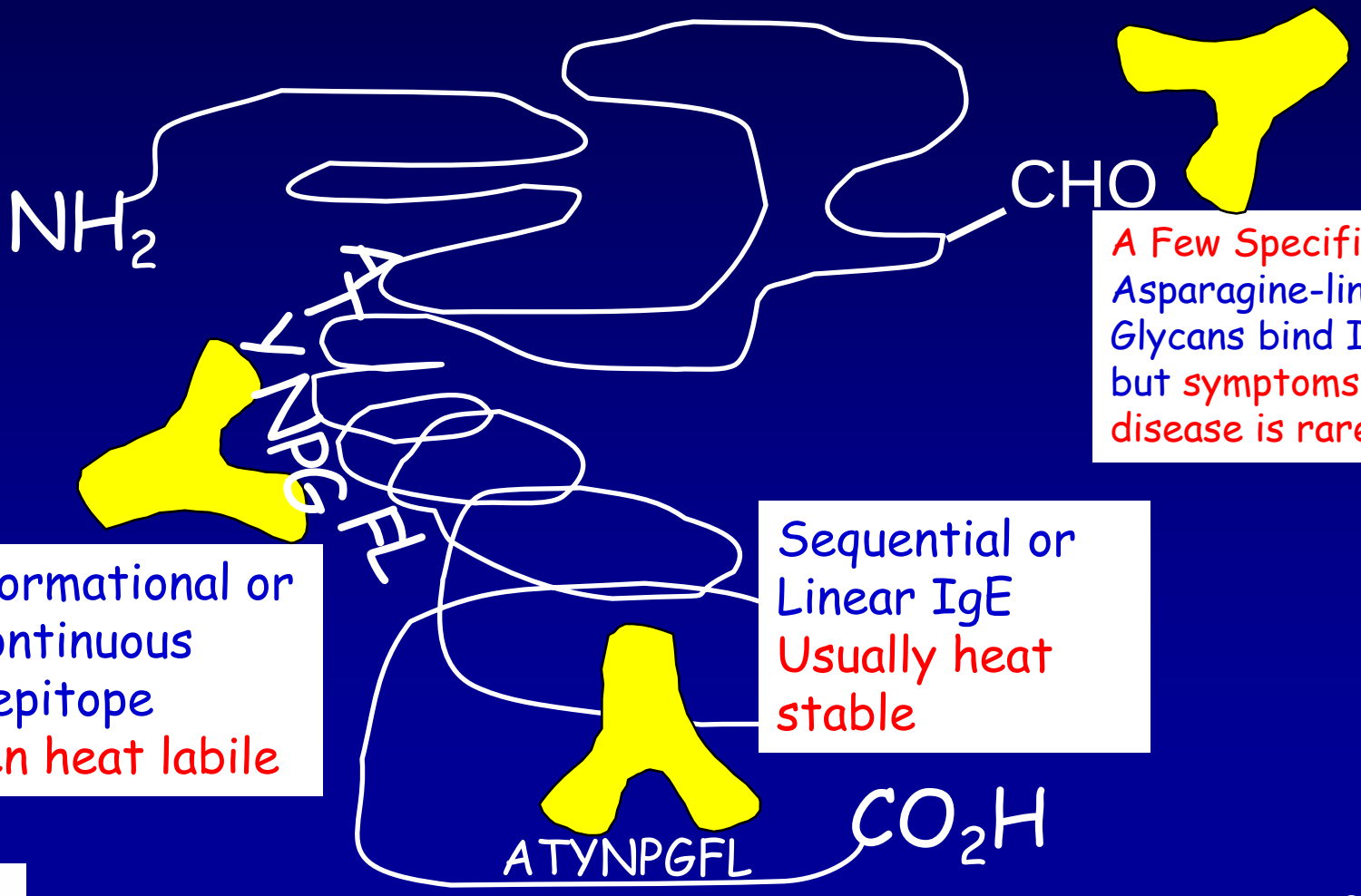
Potential IgE Antibody Binding Epitopes:

Peptides - amino acids fixed in spatial arrangement – rarely to N-linked carbohydrate



Assessing the Potential Allergenicity GMOs and Novel food Ingredients

(in order of risk)

1. Does the gene encode a protein that is known to be an allergen (or celiac)? (Evaluate source….and the protein sequence…bioinformatics, similar to an allergen?), serum IgE tests if necessary
2. Is the protein sufficiently similar to an allergen (or celiac inducing protein) to expect cross-reactions? (bioinformatics), serum IgE tests if necessary
3. Is the protein likely to sensitize and become an allergen? (e.g. stable in pepsin, abundant and stable to heating)

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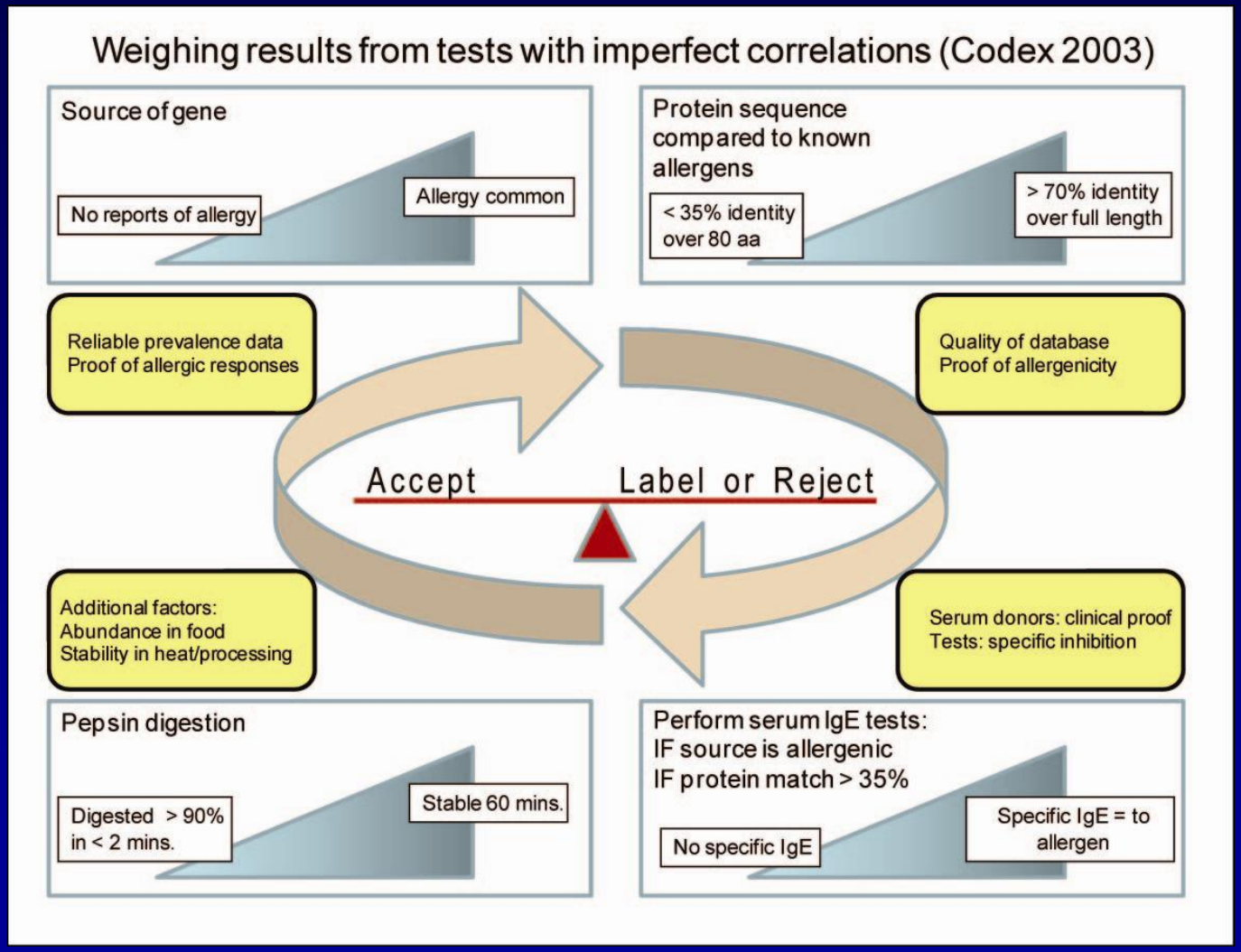
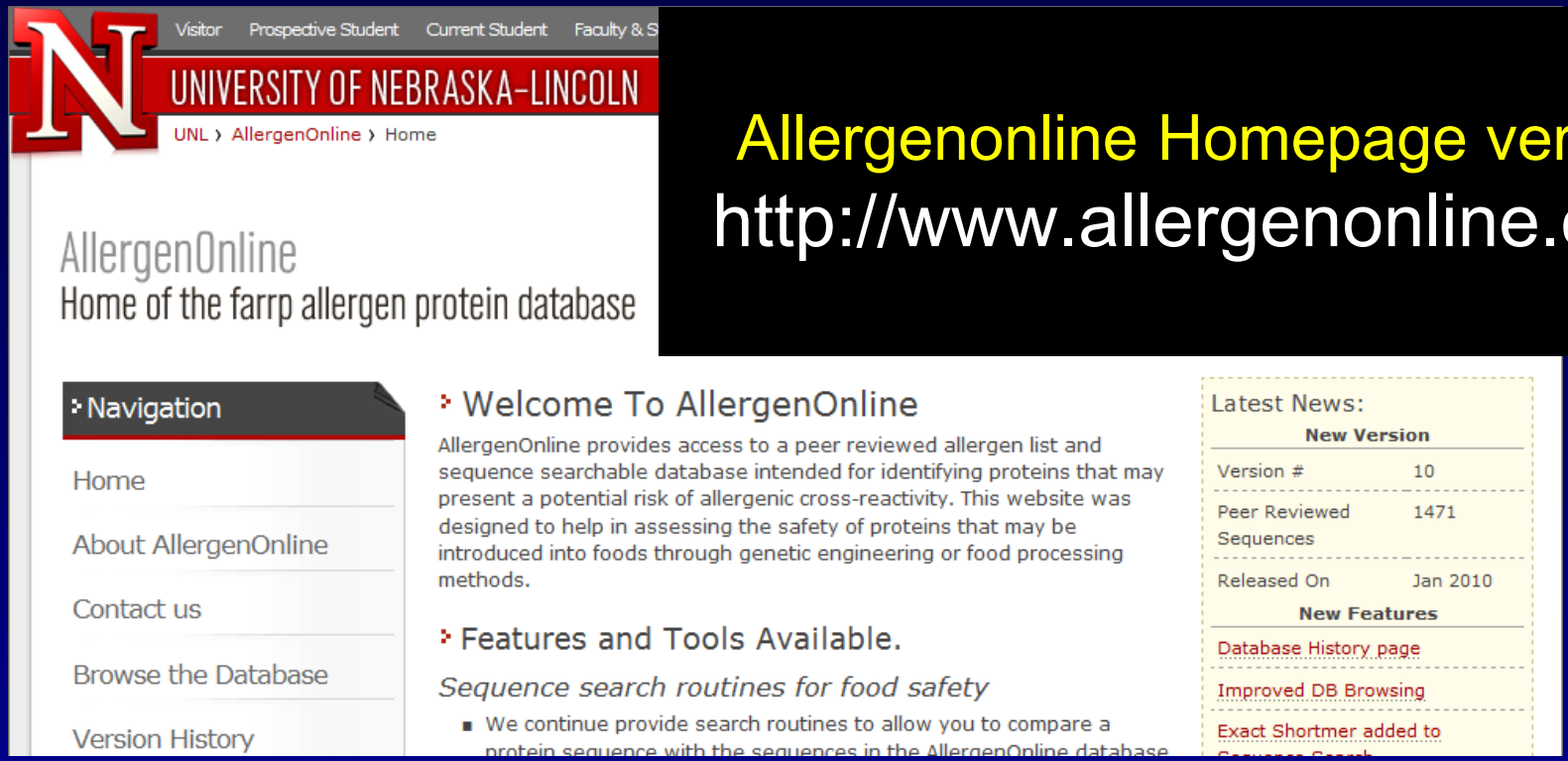
Interpretation of Codex: Goodman et al., Nature Biotech Jan. 2008

Assessing the Potential Allergenicity of GM Crops – What Makes Sense?



Sequence comparisons may predict cross-reactivity



Allergenonline Homepage ver11
http://www.allergenonline.org

1. Overall FASTA in AllergenOnline (>50% identity = structural similarity and modest to significant chance of cross reactivity)
2. FASTA scanning 80 aa window (79 aa overlap), (>35% identity = some possibility of cross-reactivity)
3. Scanning 8 amino acid identity NO PROVEN VALIDITY

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Can we predict which proteins are going to be allergens?

- Predicting B cell epitopes is imperfect
  - IgE and IgG epitopes often share the same epitopes
  - Very individualistic based on limited data
- T cell epitopes more straight-forward? but which are Th1, Th2, Th3? Or cytotoxic? And can the same epitope have multiple functions

Mapping IgE epitopes: Imperfect and Limited Data (few allergens, few patients)

Bovine beta lactoglobulin, IgE epitopes

Jarvinen … Sampson, 2001 IAAI 126:111

11 subjects over 3 year old: pooled

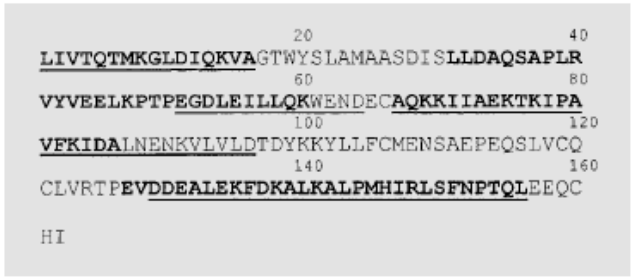


Fig. 5. Amino acid sequence of BLG. IgE binding epitopes are shown in bold. IgG binding epitopes are underlined.

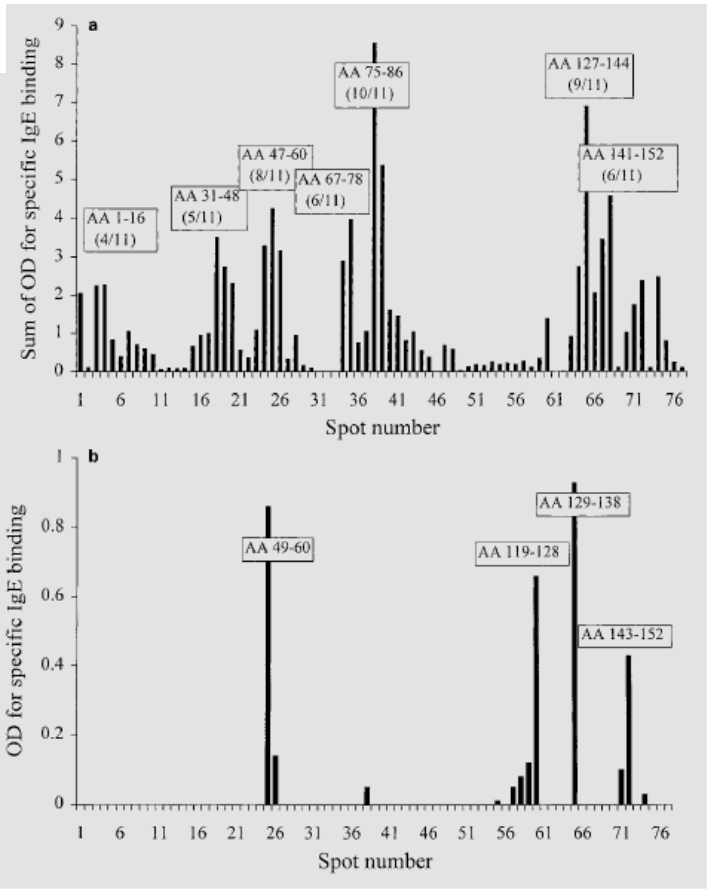
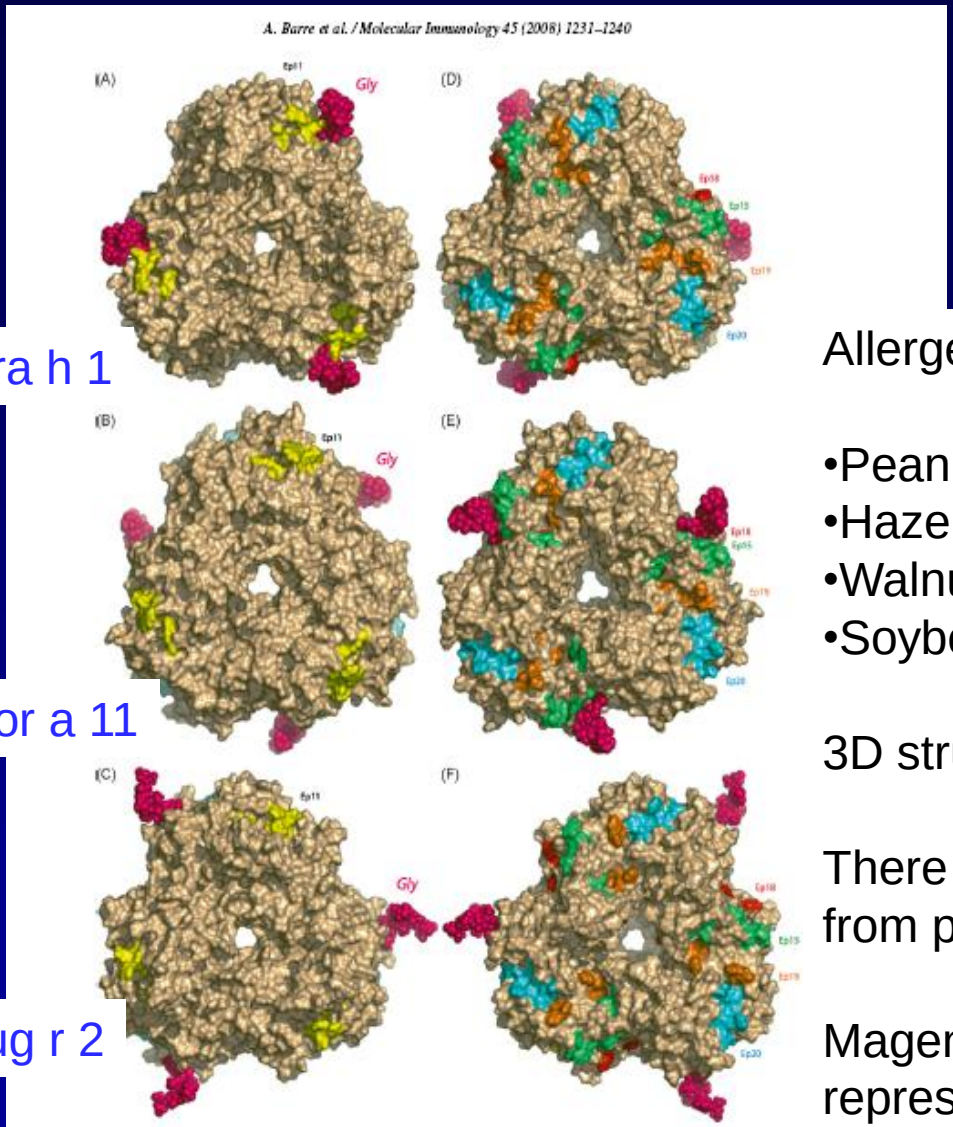


Fig. 4. Cumulative OD scores of IgE antibodies for each of the synthetic decapeptides of BLG. According to the known AA sequence, 77 decapeptides, overlapping by 8

8 subjects under 1 year old: pooled

from 8 patients with CMA who were less than 3 years of age with low levels of milk-specific IgE (**b**) were used to identify IgE binding epitopes. The boxes above the cumulative OD bars indicate the AA sequence corresponding to the allergenic epitope, and the number of patients recognizing each epitope. Control subjects had no binding to any linear epitopes.

Potential cross-reactivity CAN NOT be predicted by 3-D comparison w/o IgE



Ara h 1

Cor a 11

Jug r 2

Allergen	% Identity to Ara h 1
•Peanut Ara h 1	100%
•Hazelnut Cor a 11	34%
•Walnut Jug r 2	35%
•Soybean β-CG	51%

3D structures based on soybean β-CG

There is very weak IgE cross-reactivity from peanut to tree nuts

Magenta = carbohydrate, other colors represent putative shared IgE epitopes

Barre et al., Mol Immunology 2008, 45:1231-1240

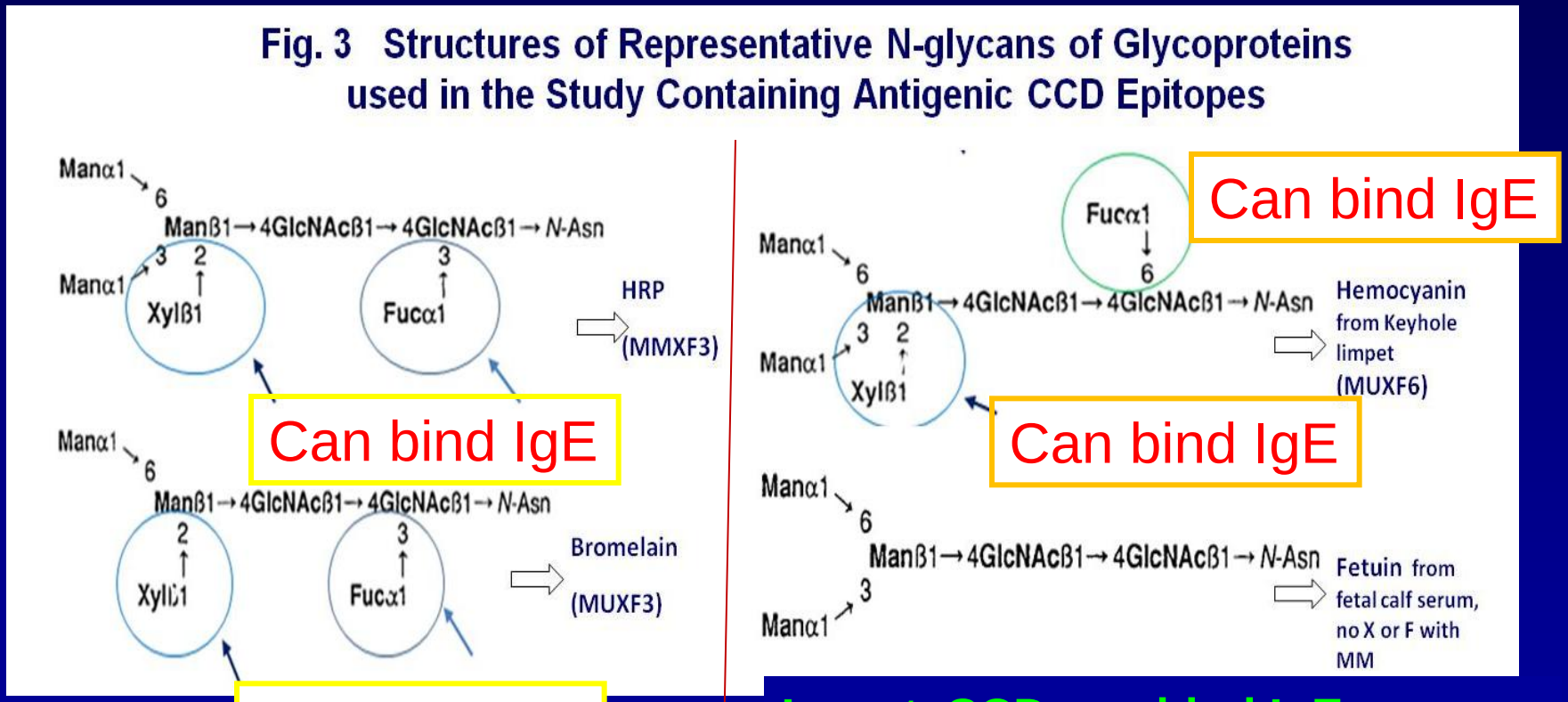
Peanut Ara h 1 Search AllergenOnline deciding which proteins to test!

Table 1a) Sequence matches to peanut Ara h 1 GI:1168390

Yellow = Direct evidence IgE binding, probable clinical cross-reactivity
Magenta = Indirect evidence IgE binding, possible clinical cross-reactivity
Green = Indirect evidence of reduced IgE binding (probably > $1/100^{th}$), no clear evidence of clinical cross-reactivity
Blue = No evidence (known) of shared IgE binding, no evidence of clinical cross reactivity

Matched Allergen	Organism	Overall FASTA3			80mer (>35%)	8mer (Identical)
Name (GI#)	Genus species	*E* value	Overlap (aa)	Identity (%)	Best % ID	Matches (#)
Ara h 1 (1168390)	Arachis hypogaea	3.1e-197	614	100	100	607
Pis s 1 (42414627)	Pisum sativum	3.9e-46	424	51.4	65	4
Len c 1 (29539109)	Lens culinaris	1.1e-68	424	53.3	63.9	4
Glycinin CG4 (256427)	Glycine max	5.9e-27	457	51.2	63.7	2
Lupinus congl (149208401)	Lupinus angustifolius	7e-57	534	49.1	62.5	1
Jug r 1 (6580762)	Juglans regia	1e-20	625	35	55.0	0
Ana c 1 (21666498)	Anacardium occidentale	3.1e-15	599	28.9	47.5	0
Cor vicilin (19338630)	Corylus avellana	1.8e-20	495	34.1	46.2	0
Ses vicilin (13183177)	Sesamum indicum	2.6e-21	561	33.2	43.9	0

Potential IgE binding to Asparagine - Linked Glycans (~1200 structures– Some bind IgE of some allergic subjects…are they allergenic?



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Animal glycan exception: Galactose α1-3 Galactose

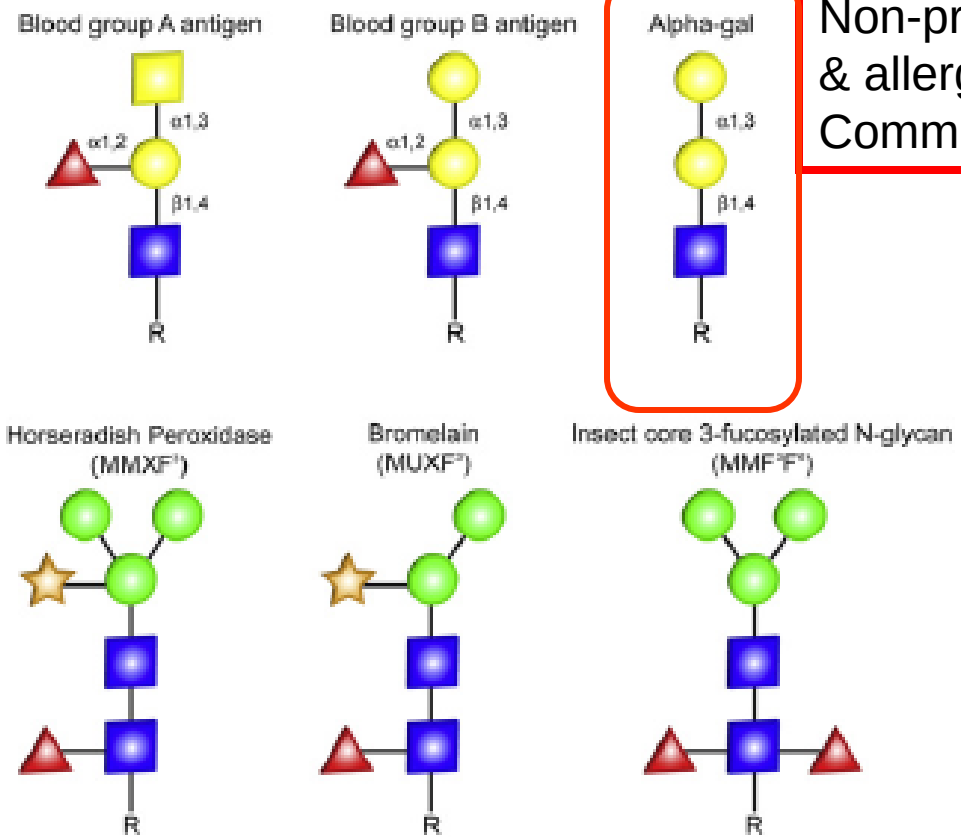
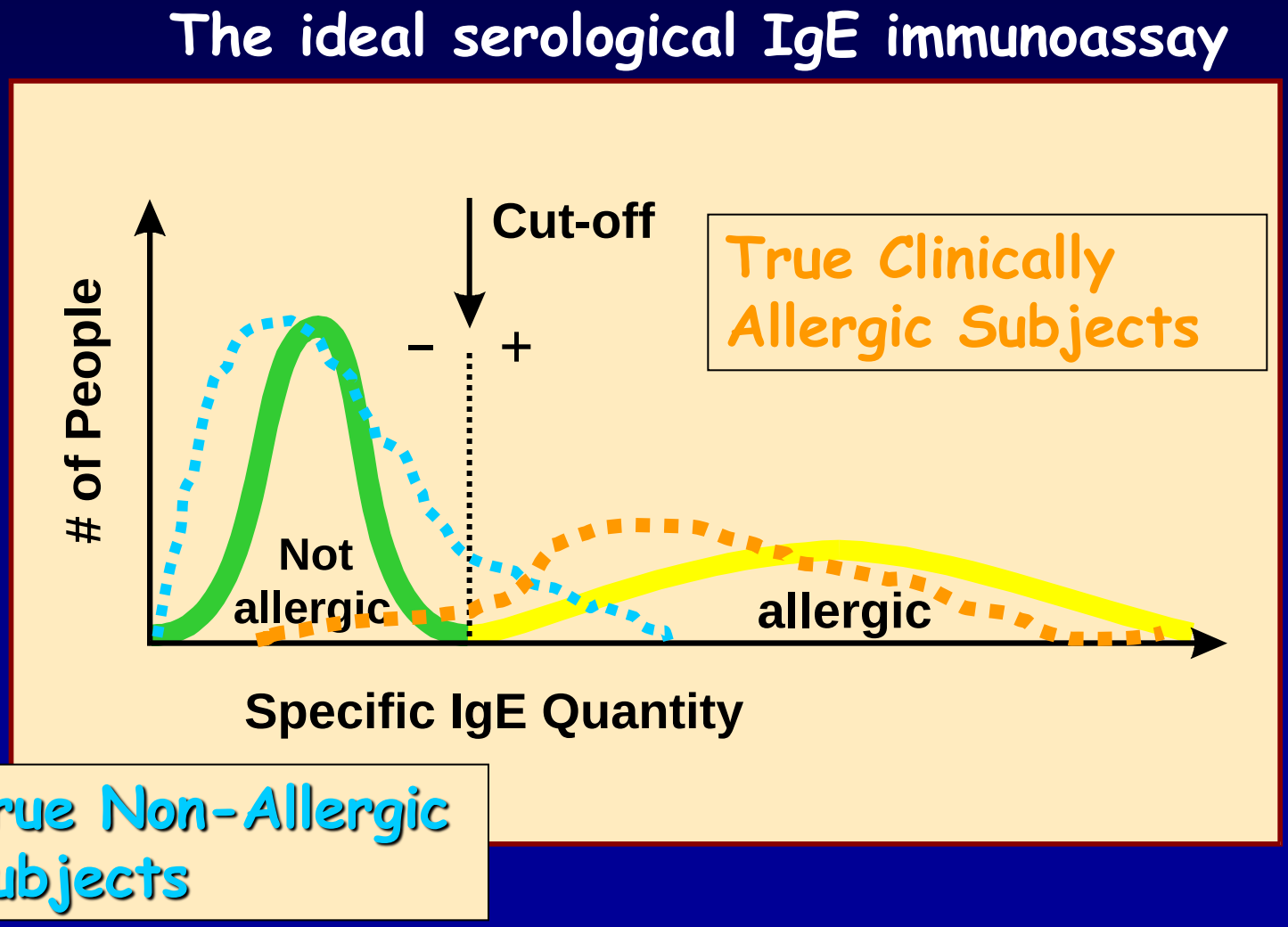


FIG 2. Comparison of representative glycans referenced in the text. The oligosaccharide structures are shown in the symbolic depiction suggested by the Consortium of Functional Glycomics, such that the *blue squares* represent N-acetylglucosamine, *green circles* represent mannose, *yellow* symbolizes galactose, while *orange squares* and *red triangles* are xylose and fucose, respectively. Note that the lack of a core fucose residue separates the structure of blood group B antigen from α-gal.

Non-primate Alpha-gal IgE binding & allergy associated with tick bites
Commins & Platts-Mills, 2009

- IgE immediate reactions to i.v. monoclonal antibody produced in CHO cells…in some tick bitten patients
- Delayed anaphyllaxis to beef, pork for similarly IgE sensitized patients
- IgG responses from xenographs of pig tissue…extra-cellular matrix glycoproteins..rejection

Serum IgE tests: must be reliable, sensitive and specific



Serum IgE Tests – based on source of gene or sequence match

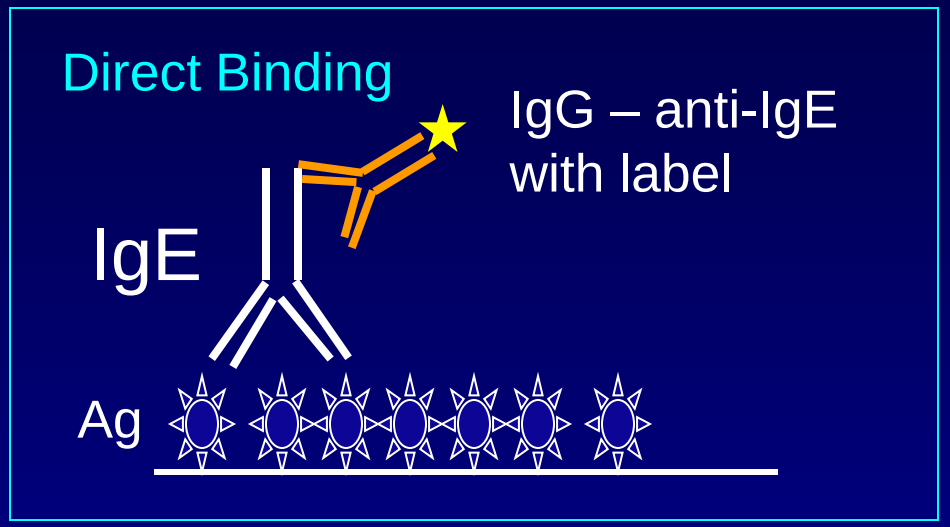
- Must be specific
- Require validation
- Positive and negative control allergic sera
- Positive and negative control allergenic proteins & extracts

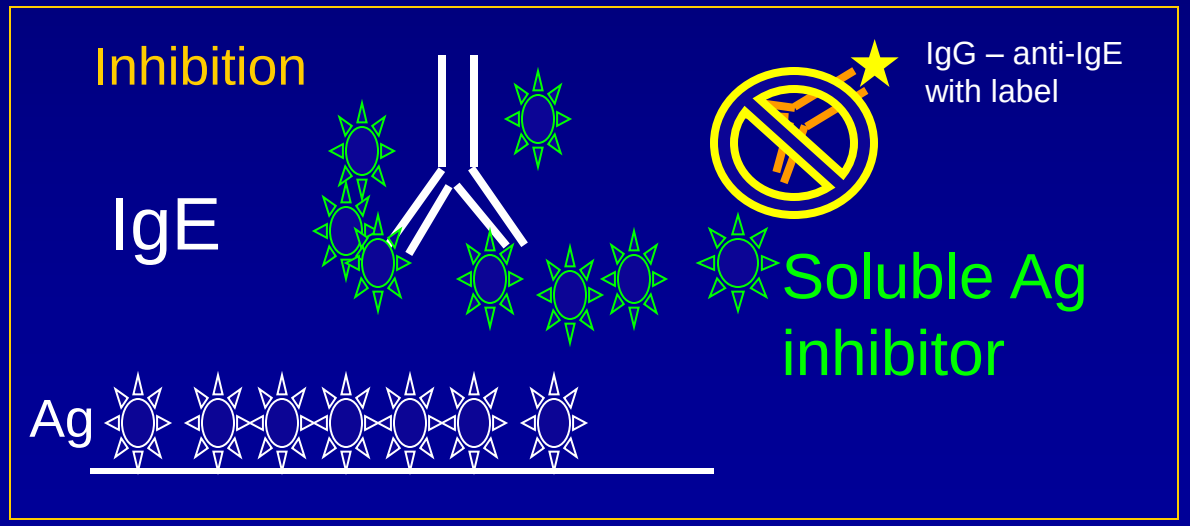
See study designed by Goodman & Vieths:

Hoff et al., 2006. Serum testing GM soy. Mol Nutr Food Res 51: 946-955

Antibody tests are needed sometimes as computer predictions do not PROVE allergy

- Dot blot ~ microarray
- Immunoblot
  - Reducing
  - Non-reducing
  - Native
  - 2-Dimensional
- ELISA
- RAST
- EAST
- Inhibition in all formats
  - Protein
  - CCD

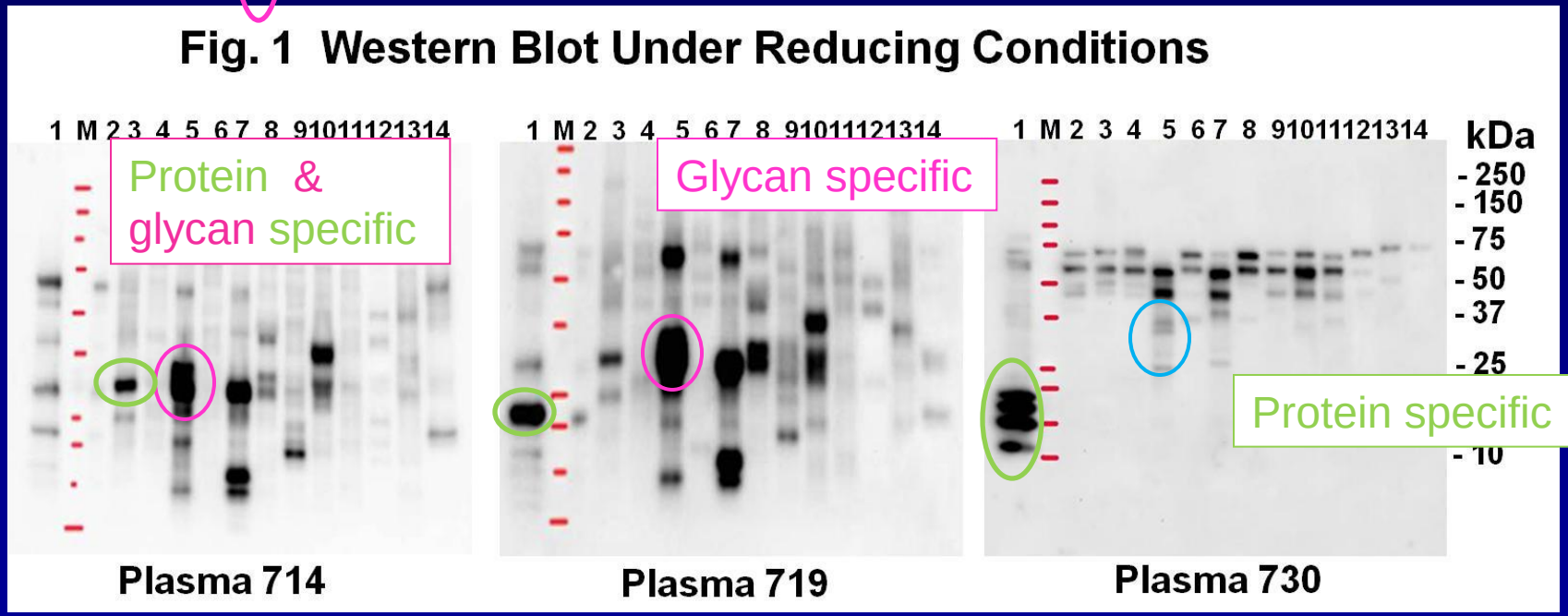




Direct IgE Western blot apparent co-sensitization or cross-reactivity? Extracts of legumes (pulses)

Protein Extracts
1: Peanut
M: Marker
2: Lupin
3: Soy
4: Green peas
5: Navy bean
6: Soja noir
7: Red Kidney bean
8: Blackgram
9:Pigeon pea
10:Lima bean
11:Cowpea
12:Fava bean
13: Rice
14: Walnut

Glycoproteins in Navy bean bind IgE from some legume allergic subjects, but it is unlikely to cause allergic rxns



Soybean Allergic

Soybean & peanut Allergic

Peanut Allergic

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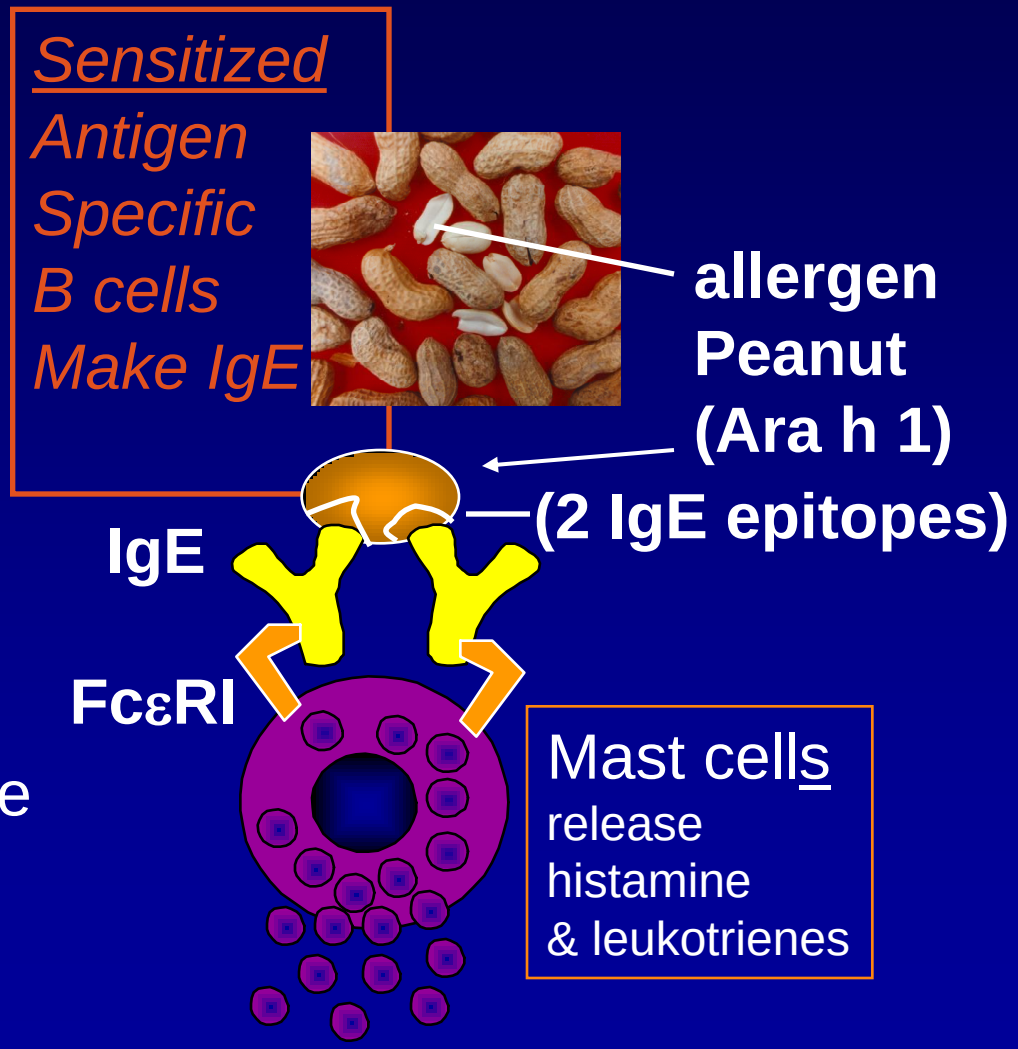
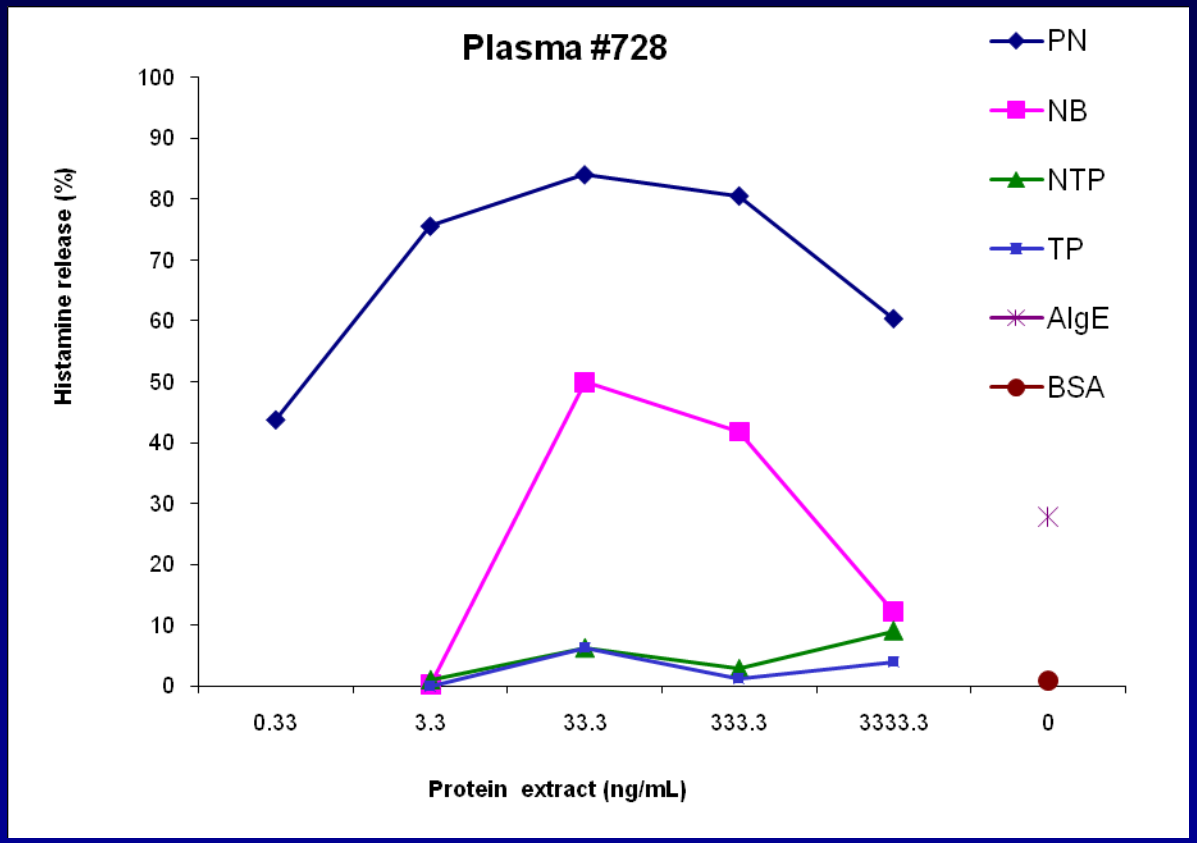
Protein-specific IgE is the key mediator in Food Allergy

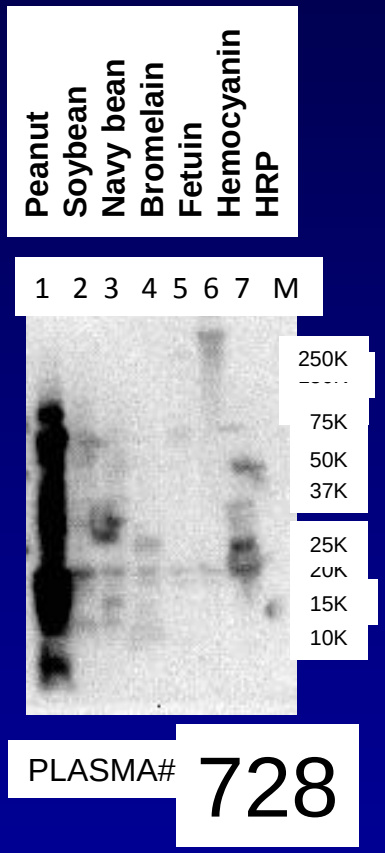
IgE binding to one epitope does NOT release histamine or cause symptoms

IgE binding to Cross-reactive carbohydrate determinants…does not (usually) cause histamine release



Histamine release assay from stripped human basophils passively sensitized with highly peanut allergic sera #728





PN = peanut….more than 100 fold stronger

NB = Navy bean

NTP = non-transgenic pea

TP = transgenic pea (aAI)

AIgE = anti-IgE control

Peanut CAPS 76 kU/L

Bean CAPS < 1 kU/L

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hLF

rh(b)LF

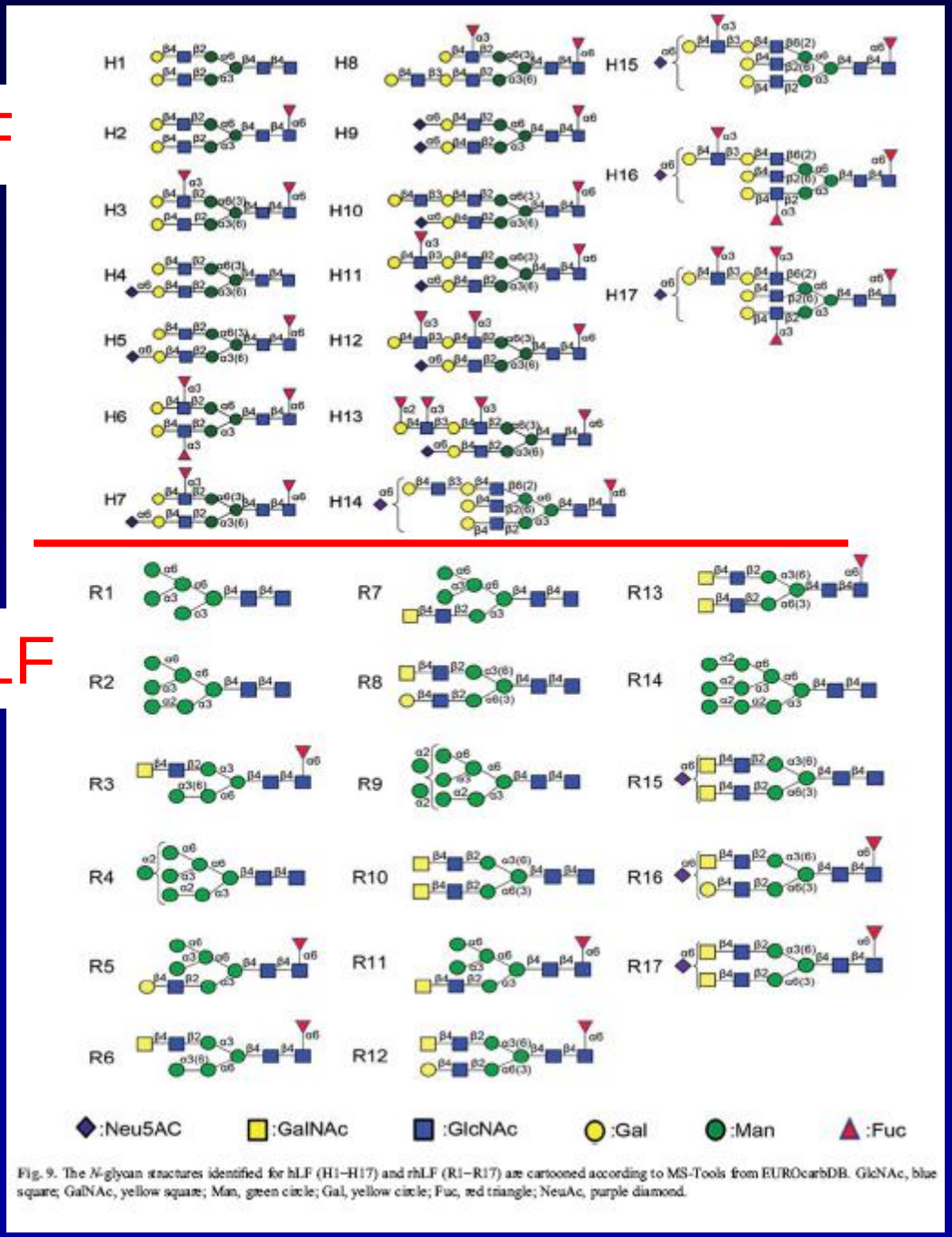

Fig. 9. The *N*-glycan structures identified for hLF (H1–H17) and rhLF (R1–R17) are cartooned according to MS-Tools from EUROcarbDB. GlcNAc, blue square; GalNAc, yellow square; Man, green circle; Gal, yellow circle; Fuc, red triangle; NeuAc, purple diamond.

Human Lactoferrin vs rhLf expressed in bovine milk Glycan structures

Yu T….Li N, 2011 Glycobiology 21(2):206-224

NO Gal alpha (1-3) gal

Therefore no demonstrable risk

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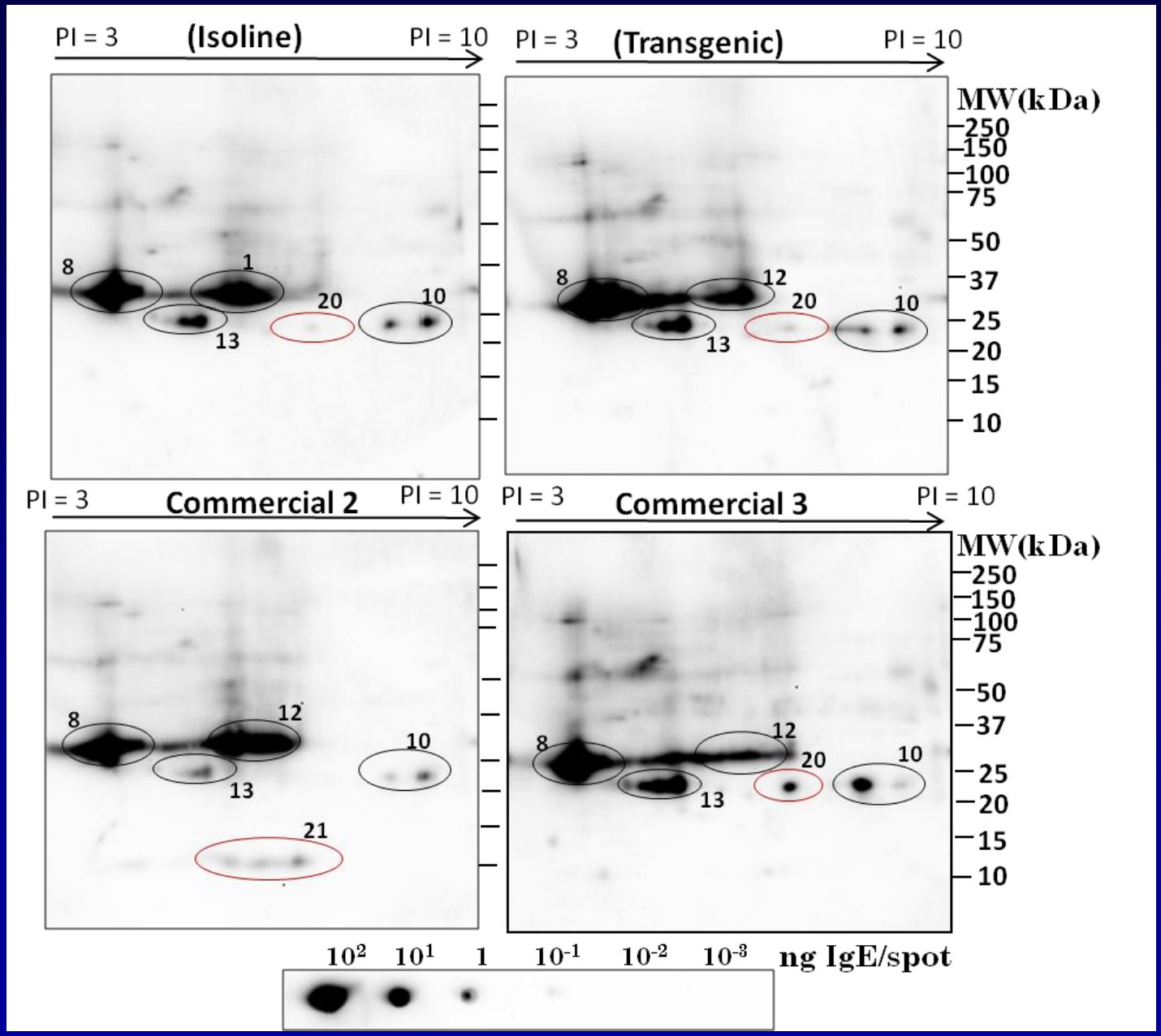
2D-PAGE and IgE immunoblots 4 soybean varieties



Serum # 714

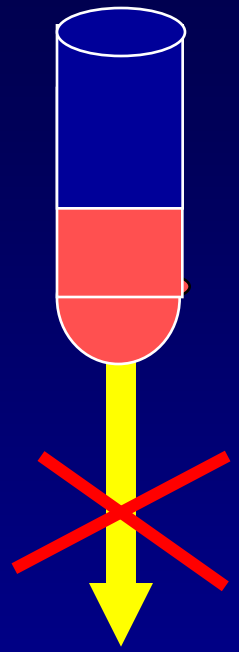
If there was no history of allergy and the sequence was not similar to a known allergen…so no serum testing (no identifiable at risk population)

Is the new protein likely to become a major food allergen?

- Hard to answer with great certainty
- But importantly, low risk….compared to:
  - Transfer of a protein that is an allergen
  - Transfer of a protein that is highly identical to an allergen and likely cross-reactive

Stability of the protein to digestion by Pepsin

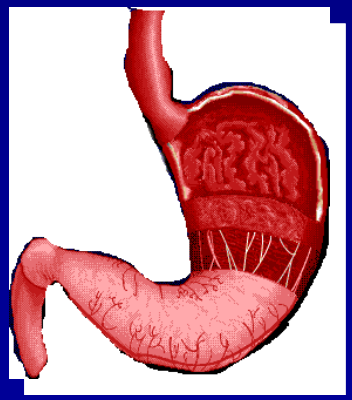
pH 1.2
or pH 2
+
Pepsin



- Assay conditions tested in a ring study
  - K. Thomas et al., Regulatory Toxicology and Pharmacology 39(2004) 87-98
- Update: Objective measurements
  - Ofori-Anti et al., Reg Tox Pharmacol (2008)

Provides a correlation for major food allergens.

This test is not meant to “mimic” real digestion



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So far NO non-human animal model has proven predictive of human allergy (for more than a few proteins)

Therefore animal models are NOT useful!

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Can Human Cell Based Assays Predict Allergy?

- Probably not at this time
- Few tests with sufficient in vivo human responses to be able to judge the cell assay results: Mixed, some success and some BIG failures
- Need validation with common strong allergens, weak and “non” allergens….or allergenic foods

Working Through Regulatory Hurdles and Food Safety Issues…No Food is 100% safe…

Sometimes the regulatory door seems shut or the walls are high…

…Politics, Economics, Philosophy…

Or scientists seeking absolute answers…

Fight for products that show benefits and have a reasonable safety profile!