Glycosylation as cause of drug hypersensitivity against protein drugs

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Hypersensitivity to Cetuximab

- 3% of Cetuximab-treated patients develop severe allergic reactions (*drug`s product label*)
- Higher rates in North Carolina, Arkansas, Missouri, Virginia, Tennessee
- 22% of patients in Tennessee and North Carolina had severe hypersensitivity reactions (*O`Neill et al., 2007*)
- 25/76 Cetuximab treated patients had a hypersensitivity to the drug (*Chung et al., 2008*)

Symptoms of type I allergy

Angioedema
Urticaria
Conjunctivitis
Laryngeal edema
Wheezing
Dizziness,collaps
Shock
nausea, vomitus

Type I-Allergy

- Genetically determinated hypersensitivity reaction

- Pathomechanism: still not fully understood, however, in case of immediate type reaction it is based mainly on the production of IgE-antibodies against *per se* harmless antigens (allergens)

Hypersensitivity to Cetuximab

•Dynamics: **Minutes after first application**

- IgE positive to Cetuximab (*Chung et al., 2008*)
- In 17/76 **IgE antibodies** against Cetuximab found in pretreatment samples
- 1/51 subjects who did NOT have a hypersensitivity reaction had anti-Cetuximab-IgE

- 15/72 control subjects in Tennessee
- 2/341 controls from Boston

- Geographical factors

Chung et al., 2008 New Engl Journal

Allergenicity

1. **Allergy depends on a sensitization period**
2. **Immune reaction**

   Already prior to therapy the patients had anti-α-Gal IgE, and a local cumulation with respect to the reaction to the therapeutic antibody cetuximab was noticed in Tennessee, Arkansas, North Carolina, Missouri and Virginia, prompting investigations on the **route of sensitization**.

Allergy to Cetuximab: Identification of the Epitope

Type delta reaction

- Chimeric mouse-human IgG1-mAb against the epidermal growth factor receptor
- Produced in a mouse myeloma cell line
- **Indication**: colorectal carcinoma squamous cell carcinoma of the head and neck

Type beta reaction

- Severe hypersensitivity reaction in **3-29%** of patients
- Anaphylactic reaction already after first application
- IgE specific for Galactose-alpha-1,3-Galactose (**alpha-GAL**)

Chung et al. 2008

Post-translational modification of a recombinantly produced molecule

- The epitope α-Gal is a disaccharide that itself is part of oligosaccharides.
- Galactose-α-1,3-galactose linkages are also found on the blood group antigen B of lower mammals.
- α-Gal = ubiquitous carbohydrate structure on cells and tissues of all mammals which are non-primates, New World monkeys, and prosimians

Epitope present on Cetuximab produced by a mouse myeloma cell line SP2/0 but not on a variant of Cetuximab produced by CHO cell line due to

Enzyme activity (i.e. α-1,3-galactosyltransferase)

Influence of the construction of biologicals

Galactose-α 1,3-Galactose highly immunogenic for humans

Sources:

- 1. Therapeutic antibodies.
  The Fab part of the heavy chain of Cetuximab is glycosylated with a set of carbohydrates on N88, including galactose-α-1,3-galactose and the sialic acid N-glycolylneuraminic acid.
- 2. Mammalian (red) meat
- 3. Cat-IgA

Allergenicity

The fact that α-Gal is present on both Fab fragments of the antibody cetuximab might favour the efficient, pairwise cross-linking of IgE on mast cells.

Glycosylation as Cause……

Infusion reaction

- IgG-Titre, seldom IgM or IgE (HACA; HAMA)
- Infusion reactions associated with high IgG-Ab-titres
- Mechanism: probably a complement activation, immune complex anaphylaxis (see dextranes, hirudin)
- Interval: 5-7 days but also 24 hrs. to 14 days

- Neutralizing antibodies: Infliximab up to 28%
- Adalimumab 6-25% of exposed patients

Allergic reactions

- IgE against alpha-GAL are the only exception (Chung et al., 2008)

- 3/11 with severe allergic reactions to Infliximab had anti-infliximab IgE and a positive skin prick test (Vultaggio et al., 2010)

So far rare IgE-detection !

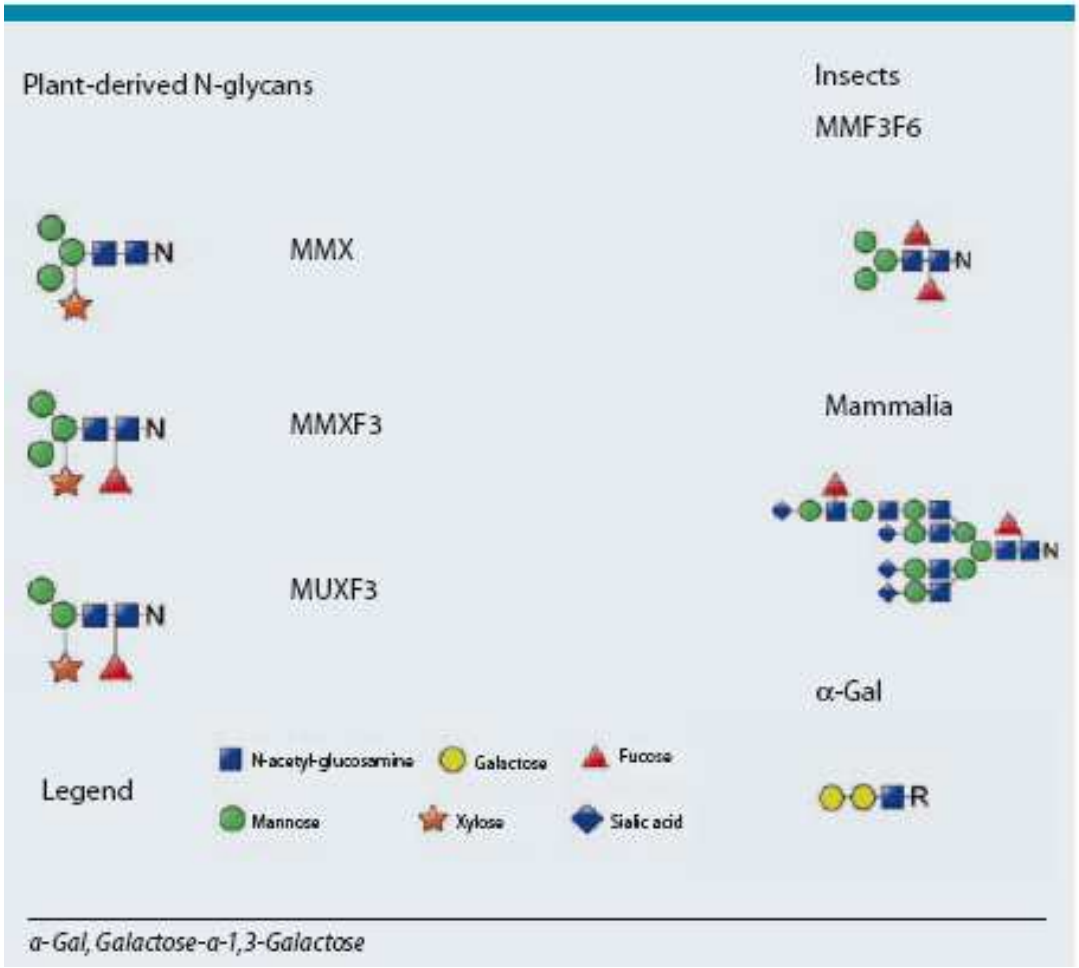
Cellular diagnostic tests non specific

Scherer *et al.*, 2010

Allergenicity of Carbohydrate Epitopes

Paradigm shift

Basically low clinical significance



Except for alphaGAL!

Jappe et al., 2013

Allergenicity

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Drug allergy – Food allergy: One Epitope

- Chimeric mouse-human IgG1-mAK against the epidermal growth factor-receptor

Glycan structure with α-GAL and sialic acid

= complex structure

Oligosaccharide Galactose-α-1,3-Galactose (α-Gal)

Single epitope:
α-Gal

- Severe hypersensitivity reactions in **3-29%** of the patients
- IgE specific for Galactose-α-1,3-Galactose (α-**GAL**)

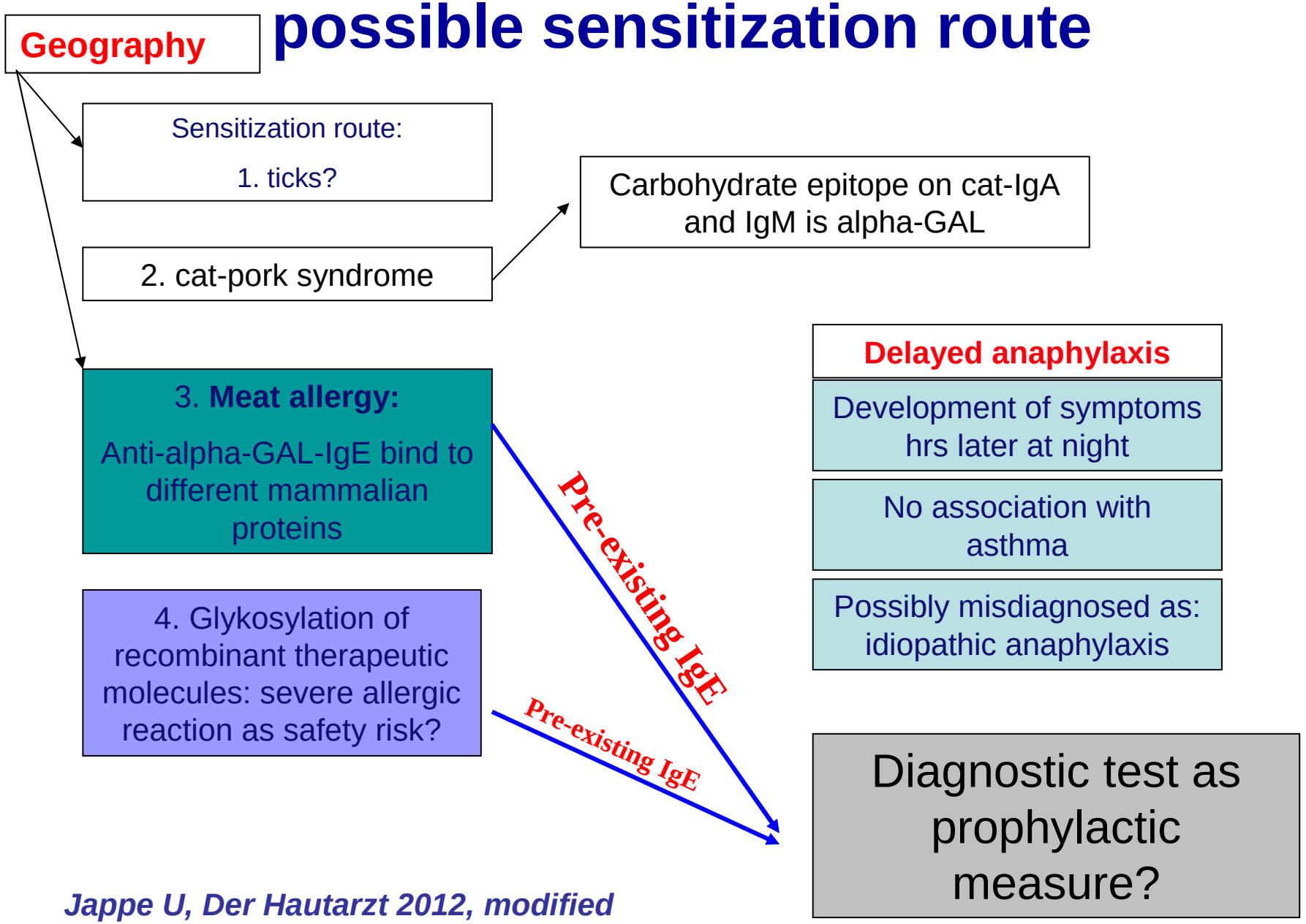
Chung et al. 2008

Recently identified allergen in red meat: no protein, but a carbohydrate epitope

Since the detection of α-Gal-specific IgE observations on meat allergy are rising in number

Chung et al. 2008, Jappe 2012

Anti-alpha-GAL-IgE: possible sensitization route



Jappe U, Der Hautarzt 2012, modified

mAb	Molecular Target		Most adverse events, mainly hypersensitivity reactions
Rituximab (MabThera)	Anti-CD 20 human constant IgG1 regions with murine variable regions of the heavy and light chain		Infusion reactions immediate type reactions 5-10% of cases, anaphylaxis, Stevens-Johnson syndrome, TEN, Urticaria in 3-14%;
Infliximab (Remicade)	TNF alpha		Infusion reaction 3,8% (4-5% der Crohn-Pat.); Hypersensitivity reactions (with anaphylaxis); autoantibody production: 6%; serum sickness 2,8% Urticaria in 6%; exanthema; single cases of type IV-reactions (Exanthema, ECT-negative, but one case of flare-up of exathema) [Vergara et al., 2002]
Cetuximab (Erbitux)	Epidermal Growth Factor Receptor		Infusion reactions, anaphylaxis (5% at first application), fever, rash, edema, anaemia, leukaemia
Adalimumab (Humira)	TNF alpha		Local reactions (6,6-15,3%) after 1-24 hrs.: 1 systemic reaction with palmoplantar pruritus and angioedema with tongue swelling [Benucci et al., 2011].; product information: allergic reactions in 1% of clinical trial patients
Certolizumab (Cimzia)	TNF alpha		Hypersensitivity reactions; lupus-like syndrome [Hussar, 2008]
Golimumab (CNTO 148)	TNF alpha		Hypersensitivity reactions, autoimmune phenomenon
Omalizumab (Xolair)	IgE-Fc-Region		Anaphylaxis (in parts delayed) (0,1%); serum sickness, systemic hypereosinophilia syndrome, Churg-Strauss-syndrome
			Scherer et al., 2010, modified

Allergy to Infliximab (Remicade)

Particuliarities

- Systemic infusion reactions:
  50% after the 1.-3. infusion

  25% after the 2. infusion
- Mostly non-specific histamin liberation, seldom allergic, but if so:

- 3/11 with severe allergic reactions to Infliximab had anti-infliximab IgE and a positive skin prick test (Vultaggio et al., 2010)

- 2 cases of anaphylaxis and successful desensitization with Infliximab [Puchner et al., 2001]; 6 cases [Brennan et al., 2009]

N-glycolylneuraminic Acid

- The most common sias are Neu5Gc and Neu5Ac
- Humans do not produce Neu5Gc
- The CMP-N-acetylneuraminic acid hydroxylase (CMAH) gene responsible for CMP Neu5Gc production is irreversibly mutated in humans
- Red meat is the richest source of Neu5Gc
- Production of recombinant glycosylated biotherapeutic agents: incorporation of the non-human sialic acid (Neu5Gc)
- But intact in non-human mammalian cells (used to produce glycosylated biotherapeutics)
- Can be taken up from animal products present in the culture medium

Significance of Neu5Gc contamination

- All humans seem to have anti-Neu5Gc antibodies
- Therapeutic glycoproteins carry various amounts of Neu5Gc

Post-translational modification of a recombinantly produced molecule

- In contrast to CHO cells murine myeloma cell lines express a greater proportion of Neu5Gc
- Only about half of Cetuximab molecules actually carry bound sias and Neu5Gc.
- This heterogeneity is typical for glycoproteins.
- Tissue accumulation of Neu5Gc together with anti-Neu5Gc IgG antibodies mediate chronic inflammation and potentially facilitates progression of disease such as cancer

Ghaderi et al., 2010

Post-translational modification of a recombinantly produced molecule

- Anti-alphaGal antibodies occur at relatively high levels in all humans

- Anti-Neu5Gc-antibody levels vary greatly (*Zangvoranuntakul et al., 2003*)

Post-translational modification of a recombinantly produced molecule

- Neu5Gc on glycans of medical agents likely originates from the production process involving non-human mammalian cell lines and/or the addition of animal derived tissue culture supplements

- All humans: spontaneous expression of antibodies against both non-human glycans: alphaGAL and Neu5Gc
- risk to increased immunogenicity to biotherapeutics carrying such non human glycan epitopes.
- *In contrast to alphaGAL, exogenous Neu5Gc can be metabollically incorporated into human cells and presented on expressed glycoproteins in several possible epitopes* (Ghaderi et al., 2012)

Unanswered Questions

In which constellation and concentration are glycan structures causative for allergic symptoms?

The association of α-Gal with proximal structures appears to be relevant for IgE-binding (Jappe, personal communication)

To the best of my knowledge, allergic reactions to biologicals have not yet been associated with IgE to Neu5Gc

The reason for delayed anaphylaxis also remains elusive.

The elucidation of sensitization routes is not yet completed.

The question if these patients should avoid red meat also is not definitely clarified.

Acknowledgement

- Dr. Sandra Minge
- Dany Warneke

- Cooperateurs:
  Priv.-Doz. Dr. Arno Kromminga
- Dr. Georgi (Ostseeklinik Damp, patient`s sera)

Manor House of the Research Center Borstel

Thank you for your attention!