



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 determined 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
- 

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 dextrans, 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)

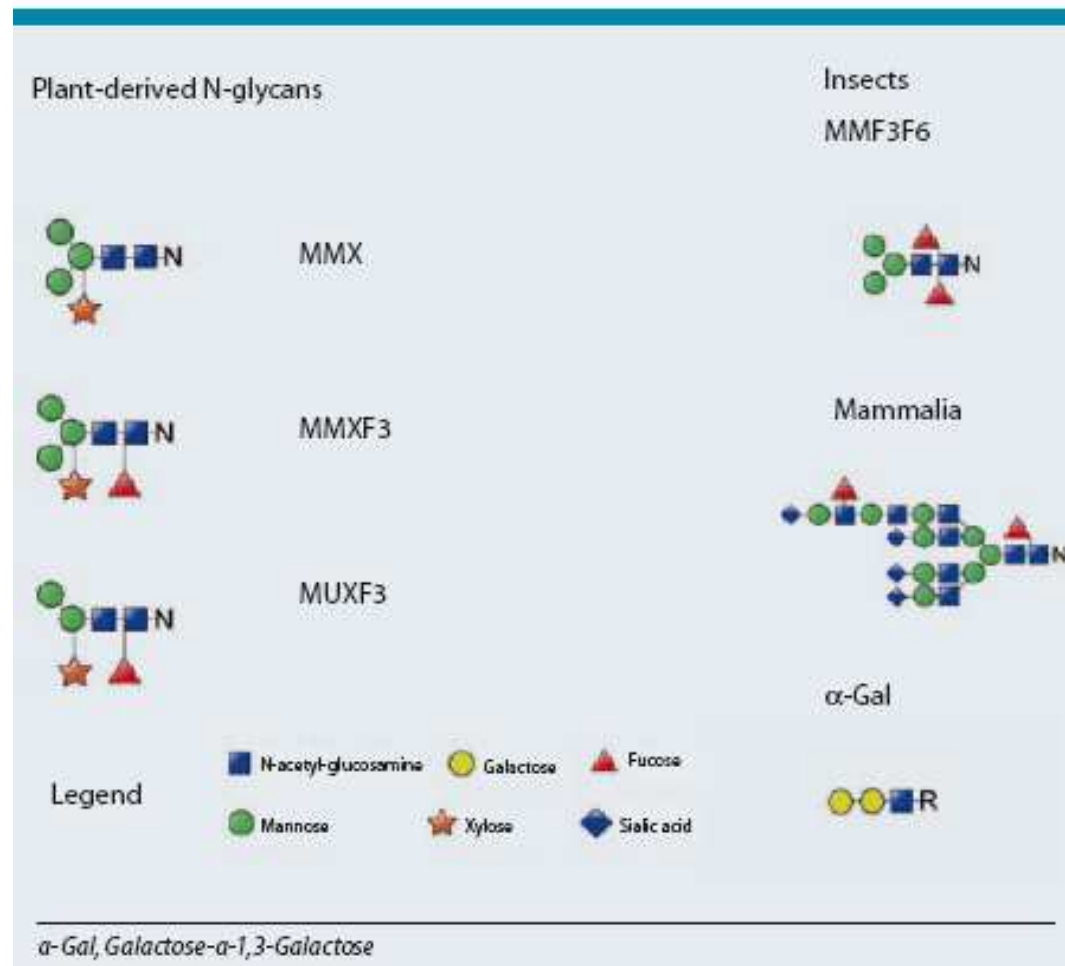
So far rare IgE-detection !

Cellular diagnostic tests non specific

Allergenicity of Carbohydrate Epitopes

Paradigm shift

Basically low clinical significance



Except for alphaGAL!

Jappe et al., 2013

Allergenicity

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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**.

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

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

Chung et al. 2008

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

Single epitope:
 α -Gal

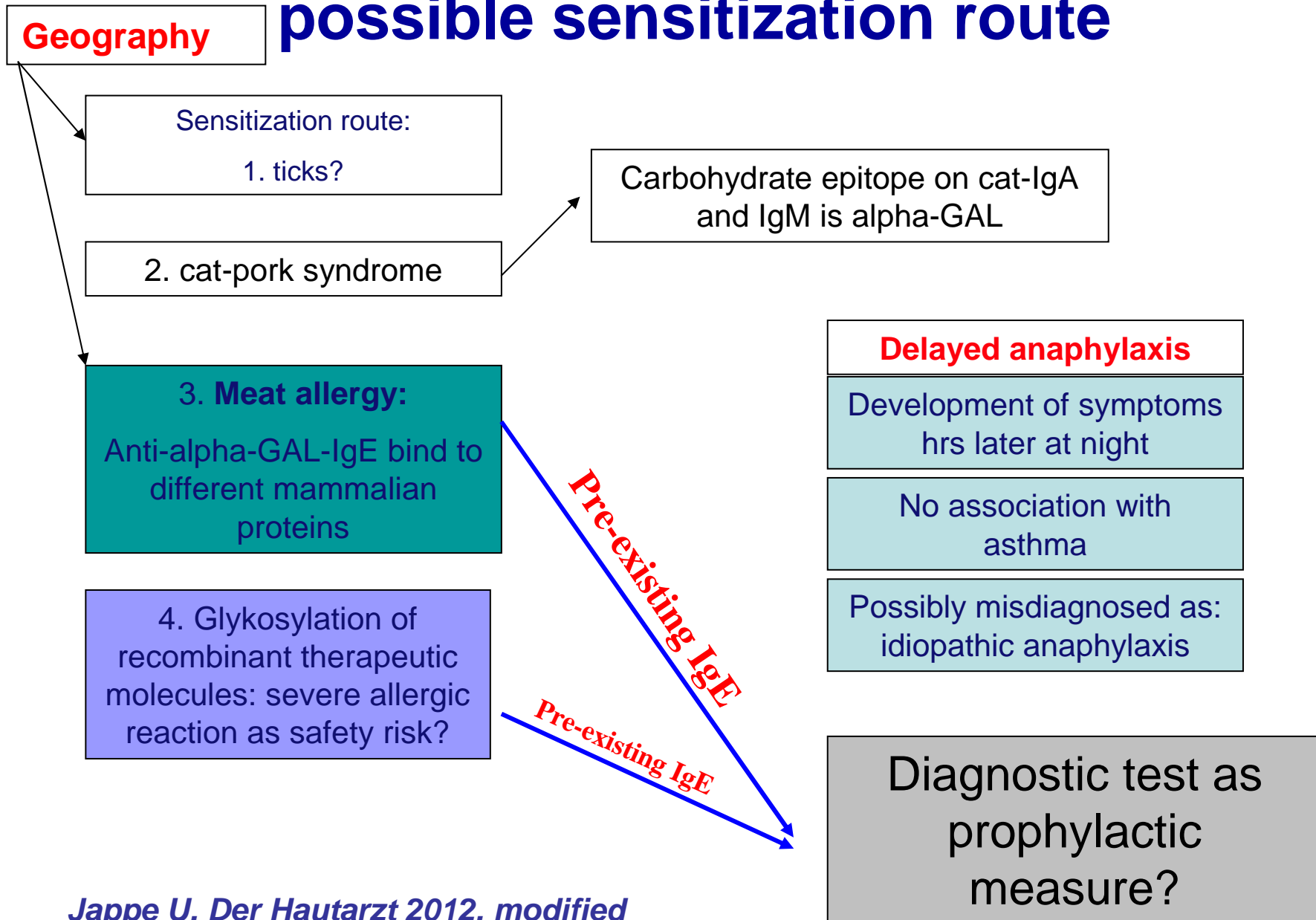
Recently identified allergen in **red meat**:
no protein, but a **carbohydrate epitope**

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



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 (Erbix)	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)

Particularities

- 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

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 metabolically 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.

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Thank you for your attention!