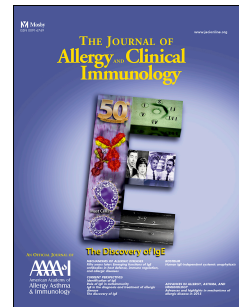


Accepted Manuscript

Glycans and Glycan-specific IgE in Clinical and Molecular Allergology - Sensitization, Diagnostics and Clinical Symptoms

Dr. Arne Homann, PhD, Dr. Gabriele Schramm, PhD, Prof. Dr. Uta Jappe, MD, MSc



PII: S0091-6749(17)30740-6

DOI: [10.1016/j.jaci.2017.04.019](https://doi.org/10.1016/j.jaci.2017.04.019)

Reference: YMAI 12787

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 3 November 2016

Revised Date: 22 March 2017

Accepted Date: 18 April 2017

Please cite this article as: Homann A, Schramm G, Jappe U, Glycans and Glycan-specific IgE in Clinical and Molecular Allergology - Sensitization, Diagnostics and Clinical Symptoms, *Journal of Allergy and Clinical Immunology* (2017), doi: 10.1016/j.jaci.2017.04.019.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Glycans and Glycan-specific IgE in Clinical and Molecular Allergology - Sensitization, Diagnostics and Clinical Symptoms

Dr. Arne Homann, PhD¹, Dr. Gabriele Schramm, PhD², Prof. Dr. Uta Jappe, MD, MSc^{1,3}

¹Division of Clinical and Molecular Allergology, Priority Research Area Asthma & Allergy, Airway Research Center North (ARCN), Member of the German Centre for Lung Research (DZL), Research Center Borstel, Borstel, Germany, ²Division of Experimental Pneumology, Priority Research Area Asthma & Allergy, Research Center Borstel, Borstel, Germany, ³Interdisciplinary Allergy Outpatient Clinic, University of Lübeck, Lübeck, Germany

Corresponding author:

Prof. Dr. Uta Jappe, MD, MSc

Division of Clinical and Molecular Allergology

Research Center Borstel

Parkallee 35

23845 Borstel

Phone +49 4537 188 7406

Fax +49 4537 188 6030

Email ujappe@fz-borstel.de

Keywords

cross-reactive carbohydrate determinants (CCD); alpha-Gal; anti-glycan IgE; anaphylaxis; clinical allergology; molecular allergology; Schistosoma; parasite immunomodulation

Abstract

Glycan-specific IgE antibodies are cross-reacting with highly similar or even identical carbohydrate structures on a variety of different natural allergens, the so-called cross-reactive carbohydrate determinants (CCDs). In clinical practice, CCDs often interfere with the specificity of *in vitro* allergy diagnostics, thus impairing allergy therapy decisions for individual patients. Strikingly, these IgE antibodies directed against CCD often do not cause clinically relevant allergy symptoms. Opposed to that, the IgE-binding glycan allergen galactose- α -(1,3)-galactose (α -Gal) is associated with IgE-mediated delayed anaphylaxis in meat allergy. The reason for this discrepancy is not known. The discovery of α -Gal stimulated new discussions and investigations regarding the relevance of anti-glycan IgE for allergic diseases. In this review, the impact of glycans and glycan-specific IgE on sensitization to allergens and allergy diagnosis is described. As parasite infections elicit a similar immunological environment as allergic diseases, the association of glycan-specific antibodies against parasite glycoproteins with glycan structures on allergens are discussed.

Abbreviations

α -Gal	galactose- α -(1,3)-galactose (IUPAC: α -D-galactopyranosyl-(1,3)- α -D-galactopyranose)
AAM	alternatively activated macrophage
APC	antigen-presenting cell
B _{reg}	regulatory B cell
CCD	cross-reactive carbohydrate determinants
CD62E	E-selectin
CFG	Consortium for Functional Glycomics
CTX	cetuximab
DAMP	danger-associated molecular patterns
DC	dendritic cell
DC-SIGN	dendritic-cell-specific intercellular-adhesion-molecule-3-grabbing non-integrin
EMR2	epidermal growth factor (EGF)-like module-containing mucin-like hormone receptor-like 2
ER	endoplasmatic reticulum
Fab	fragment, antigen binding
Fc	fragment, crystallizable
Fuc	L-fucose
Gal	D-galactose
GalNAc	D-N-acetyl galactosamine
GlcNAc	D-N-acetyl glucosamine
GPCR	GTP- (G-) protein-coupled receptor
HRP	horseradish peroxidase
HSA	human serum albumin
Ig	immunoglobulin
IPSE	IL-4-inducing protein from schistosome eggs
IL	interleukin
LacNAc	N-acetyl lactosamine
LDN	LacdiNAc, di-N-acetyl-lactosamine
Le ^x	Lewis X
LPS	lipopolysaccharide
Man	D-mannose
MC	mast cell
(M)MR, CD206	(macrophage) mannose receptor
MGL	macrophage galactose lectin
MHC	major histocompatibility complex
Neu5NAc, NANA	N-acetyl neuraminic acid
Neu5Gc, NGNA	N-glycolyl neuraminic acid
PAMP	pathogen-associated molecular pattern
PBMC	peripheral blood mononuclear cells
SIGLEC	Sialic-acid-binding immunoglobulin-like lectin
SIT	(allergen-) specific immunotherapy
TLR	toll-like receptor
Th2	T helper cell, type 2
TNF- α	tumor necrosis factor α
T _{reg}	regulatory T cell
Xyl	D-xylose

Outline

In this review, we define patient groups (Fig. 1) with IgE specific for distinct glycans and associated with clinically relevant allergy symptoms. Target structures of anti-glycan IgE from plants, mammals and parasites are introduced (Fig. 2 and 3) and immunological mechanisms which either lead to allergy symptoms or tolerance are characterized (Fig. 4). Mirroring allergy processes, helminths induce a Th2 immune response including IgE which is directed against the glycan portion of distinct parasite glycoproteins (Fig. 5). This parasite model can contribute to clarifying functional aspects of glycans in human inflammatory processes (Figure 6).

1 Definition of patient groups – Diagnostic challenges in assessment of peptide- and glycan-association of allergen-specific IgE

In order to shed light on the situation of patients with or without anti-glycan IgE and with or without associated clinical symptoms, subgroups of patients are defined and described in more detail that differ in their IgE sensitization profile and allergy status (Fig. 1). Two main groups of individuals with IgE are defined: Group A with anti-glycan IgE and group B with IgE against the peptide moiety of an allergen. Whereas patients of group B are relatively well-known in practice and can generally be diagnosed by the available test systems, group A is challenging in terms of correct diagnosis due to the cross-reactivity of IgE against CCD. In this review, patient group A1 is defined as individuals with anti-glycan IgE and clinically relevant allergy symptoms, e.g. IgE against α -Gal. Patients from group A2 have anti-glycan IgE but no or minor allergy symptoms. This anti-glycan IgE is directed mostly against CCD. In terms of anti-glycan IgE diagnostics, recently, additional to CCD diagnostics, commercial anti-glycan IgE detection systems are provided for IgE against α -Gal (galactose- α -(1,3)-galactose). Consequences of the anti-glycan IgE test results and their impact on the therapy for allergy patients are discussed in this review. Comparison of the two groups A1 and A2 with anti-glycan IgE shall start a discussion considering the impact of IgE-binding glycans in allergy in the light of their biological function.

2 “classical” CCD

2.1 Structure-functional aspects of “classical” CCD

Allergens from very different sources like invertebrates (insect venom), plants (especially food) and also parasites such as helminths exhibit common carbohydrate structures (Fig. 2 and 3). These glycans with known IgE-binding properties in this review are termed “classical CCDs”. Although the general structures of CCDs are quite similar to the human N-glycan core, CCD glycans have two characteristic features: 1) non-human modifications like foreign monosaccharides or linkage types and 2) aberrant glycosylation pattern with regard to human glycans. The non-human IgE-binding monosaccharide unit of classical CCDs is xylose linked β -(1,2) to the first

mannose of the trimannosyl motif in the N-glycan core region (Fig. 2). The non-human linkage type that binds IgE refers to the fucosyl residue, which is linked α -(1,3) to the first N-acetyl glucosamine (GlcNAc) in the N-glycan core region (Fig. 2). While xylose is a non-human monosaccharide and therefore is immunogenic, α -(1,3)-linked fucose on CCD generally is a human monosaccharide, but not α -(1,3)-linked in the N-glycan chitobiose core region (in humans, N-glycan core fucose is linked to position 6 of the first GlcNAc unit). As part of the CCD molecules MUXF and MMXF, these immunogenic fucose and xylose moieties have been identified as responsible for IgE-binding and IgE cross-reactivity.^{1,2} The MUXF CCD type is the most prominent example for the immunogenicity of aberrant glycosylation. Here, the 3-branched mannose unit of the N-glycan trimannose core is deleted resulting in a structure that is not part of human glycans. Pro-allergic immunological effects of allergens in some scenarios depend on their glycosylation and interaction with human carbohydrate-binding cell receptors.³⁻⁶ For example, the major peanut allergen Ara h 1 with the CCD MMX as dominant glycan motif is a ligand of the dendritic cell (DC) receptor DC-SIGN and acts as a Th2 adjuvant.^{7,8} Mimicking this effect, it has been shown that parasite glycans are structurally similar to CCD and also induce Th2 responses.⁹ These aspects of the biological relevance of glycans will be addressed later in this review.

2.2 Diagnostics and pathophysiology of IgE against “classical” CCD

In order to address diagnostic uncertainties for patients with regard to the clinical relevance of anti-glycan IgE (patient group A, Fig. 1), the following approaches have been accepted: One approach is to include classical CCDs (e.g. MUXF from bromelain and both, MMXF and MUXF from HRP) in the diagnostic platform in order to differentiate the IgE sensitization pattern in polysensitized patients for better discrimination between primary sensitizing allergen and mere cross-reactivity.^{10,11} However, there is no guideline for the interpretation of a positive or negative anti-CCD IgE test result and, thus, the (unclear) consequences of a potentially positive result regarding treatment decisions (group A, Fig. 1). An alternative approach is to exclude certain CCD-binding IgE from the IgE-detection test system by removing them beforehand from the patient sera by pre-incubation with a commercially available molecule containing CCD of the MUXF-type.¹² However, not all types of cross-reacting carbohydrates are available for allergy tests at present. In earlier studies, deglycosylation of diagnostic allergens by periodate treatment was performed, however, this as well as methodically laborious inhibition assays are not established as routine tests and, therefore, are not commercially available. Concerning the number of patients sensitized to CCD, our investigations showed a prevalence of up to 72% of anti-CCD-IgE-positive insect venom allergic patients.¹³ The prevalence of anti-CCD IgE in patients with food allergy varies between 10-50% in the literature depending on the study and the analyzed food (zucchini, celery, carrot, and tomato).¹⁴ Interestingly, in most patients IgE against CCD seem to cause no clinically relevant allergy symptoms.¹⁵⁻¹⁸ However, as some CCD-containing allergens are able to differentially activate basophils, IgE against CCD motifs at least has to be considered as a risk factor for allergic symptoms.^{19,20} It remains to be

determined if a subgroup of individuals with anti-CCD-IgE is underdiagnosed and suffers from allergy symptoms derived and mediated by glycan-specific IgE (Fig.1, group A2).¹⁵ Among our patients allergic against insect venom, there were 3 individuals with systemic reactions whose serum showed 100% inhibition of bee and wasp IgE via HRP, a CCD-screening allergen containing CCD of the MUXF- as well as MMXF-type. Although the detailed molecular allergy diagnostic tests with single species-specific venom allergens was not available at that time, the complete (up to 100%) inhibition of anti-bee- and anti-wasp-venom-IgE by the CCD-containing allergen HRP alone is highly indicative for the clinical relevance of the anti-CCD-IgE in these 3 cases. If there was additional species-specific IgE present, there would have been no complete inhibition.^{13,21} As recombinant allergens are used in commercial diagnostic assays, it is very important to consider the differing glycosylation pattern of these recombinantly produced allergens.²⁰ Here, the organism that is used as expression system is crucially important. For example, allergens from *E. coli* can generally be considered as non-glycosylated, whereas allergens produced in the yeast *Pichia pastoris* carry predominantly high mannose-type N-glycans (Fig. 3). Accordingly, allergens derived from plants, insect cells or mammalian cells carry glycans of varying complexity (Fig. 3). The details of this glycan complexity strongly depends on the specifications of the manufacturing process. This difference in the glycosylation pattern of recombinant allergens compared to natural allergens may lead to the known phenomenon of false positive results as in the case of classical CCDs. But also false negative test results can be the consequence: if the glycan on an allergen is crucial for IgE binding and additionally has a biological activity leading to allergy symptoms.

3 α -Gal

3.1 Structure-functional aspects of α -Gal

The glycan IgE epitope α -Gal has been identified a few years ago as being responsible for anaphylactic reactions in meat allergy and allergy against the therapeutic monoclonal antibody cetuximab.^{22,23} This was the first proof that glycans play an important role in allergy development and that glycans can cause fatal anaphylaxis. The structure of α -Gal basically is simple: it is a disaccharide consisting of two galactose monosaccharides which are linked α -(1,3). However, anti- α -Gal IgE antibodies (and also anti- α -Gal IgG) have a higher affinity to the trisaccharide consisting of the two galactose units plus the following N-acetyl glucosamine which is part of protein N-glycosylation.²⁴ It remains to be determined how the vicinity of this motif influences the anti-glycan antibody binding. On N-glycans, there are several different glycan motifs present in the vicinity of α -Gal, as these glycans are usually branched to different degrees. On glycoproteins there are also different peptide motifs located in the vicinity of α -Gal which may influence the binding properties of antibodies against the glycan. The α -Gal motif is common in most mammals, but immunogenic in humans.²⁵ The enzyme responsible for the synthesis of this motif, the α -galactosyltransferase, is mutated in humans.²⁵ Of note, an α -linked galactose in a human subpopulation is found as part of the blood group B

motif (Fig. 2). However, studies showed that there is no clear evidence for an association of tolerance against α -Gal in individuals with blood group B,²⁴ probably because one galactose unit of blood group B is fucosylated and thus the glycan structure is not identical to α -Gal (Fig. 2).

3.2 Pathophysiology and diagnostics of IgE against α -Gal

In most cases – and also in the general view of allergologists – IgE against classical CCD (Fig. 2) does not cause clinically relevant allergy symptoms. This paradigm shifted in 2008 when the first anaphylactic reaction mediated by anti-glycan IgE to the therapeutic monoclonal antibody cetuximab (CTX) was identified.²³ This anti-glycan IgE is specific for α -Gal, as demonstrated by analysis of neoglycoprotein (e.g. HSA- α -Gal) conjugates.²⁴ With the identification of α -Gal as the causative allergen, a conceptually intriguing IgE-mediated immune reaction to this glycan was characterized: the chimeric therapeutic antibody CTX contains an N-glycosylation site in its variable F_{ab} region additional to the conserved IgG₁-specific Fc glycosylation site (Fig. 4C). Analysis of the N-glycan revealed a high content of α -Gal.^{26–28} Moreover, the bivalent and flexible nature of the α -Gal presentation by the two F_{ab} arms of CTX is an ideal situation for efficient IgE cross-linking, resulting in the release of cytokines and pro-inflammatory mediators by mast cells (MC) and basophilic granulocytes (basophils) (Fig. 4B and C). Since the majority of CTX-specific allergic reactions occur at first application it was mandatory to search for the sensitization route, as obviously this could not have been CTX itself. As a result, it has been discovered that red meat is a trigger for α -Gal-mediated anaphylaxis, the route of sensitization probably being tick bites.^{29–31} This was a new perception in so far as the triggers for meat and innard allergy on the molecular level had not been identified for years. Until 2008, the non-glycosylated bovine serum albumin (BSA) was considered to be the major meat allergen (Bos d 6). However, extract-based routine allergy *in vivo* as well as *in vitro* diagnostics remain negative in a lot of patients reporting allergy symptoms after meat consumption which is most probably due to the low α -Gal content. Having identified the glycan allergen α -Gal on the molecular level, now more diagnostic tools become commercially available for routine allergy *in vitro* diagnostics and, thus, more and more patients with meat allergy can be diagnosed correctly. Currently, three α -Gal-carrying proteins are registered as allergens by the WHO/IUIS subcommittee: Cetuximab (Hom s / Mus m Cetuximab), bovine thyroglobulin (Bos d TG) and bovine carbonic anhydrase (Bos d CA). A lot of α -Gal-associated meat-allergic patients were underdiagnosed in the past due to the lack of carbohydrate- (α -Gal-) specific IgE detection methods. Today, CTX and commercially available bovine TG are used as α -Gal-containing test molecules, both with advantages and disadvantages: For both antigens, the exact α -Gal content is not known and, furthermore, can vary from batch to batch.^{26,32} Bovine TG is a non-human protein and, therefore, contains immunogenic peptide sequences additional to the IgE-binding glycans. CTX is a chimeric IgG₁ antibody which contains murine peptide sequences in its variable region (Fig. 3 B). IgE antibodies binding to IgG (IgE rheumatoid factors) interfere with the diagnostics and serum anti-CTX IgG₁ antibodies cannot be detected due to isotype/subclass interference.

Moreover, the detection of anti-CTX IgG₄ antibodies is also not possible as IgG₄ binds to immobilized IgG₁,³³ and own observations). Taken together, there are a lot of glycan-specific diagnostic challenges when the results for IgE against α -Gal are interpreted.

4 Pathophysiology of clinical cases potentially associated with IgE directed against glycans

Clinically relevant allergic reactions are also observed against glycan-containing additives to drug formulations. In this regard, α -Gal also plays a part since patients with severe allergic reactions to gelatin-containing medications and food possess anti- α -Gal IgE.³⁴ However, cross-reactivity between red meat and gelatin-containing products may be not directly comparable due to differentially glycosylated protein content. Another IgE-mediated reaction against a glycan-containing drug additive is the adverse reaction to mannitol. In 2013, a fatal reaction to intravenous application of thymoglobulin that contained the additive mannitol was described.³⁵ The patient developed anaphylaxis immediately after injection and died. *Post-mortem* allergy diagnostics that in general includes anti-natural rubber latex-IgE detection in cases where the application of treatment induces fatal reactions, revealed IgE specific only for classical CCD of the MUXF-type but not against natural rubber latex extract. The authors hypothesized that the IgE-mediated mechanism of the anaphylactic reaction was due to anti-CCD-IgE directed against the drug additive mannitol and not to the active drug thymoglobulin derived from rabbits.³⁵ However, IgE against other glycans, e.g. α -Gal which is present on rabbit glycoproteins and may be responsible for the fatal allergic reaction, was not tested in this case.

5 Triggers for anti-glycan IgE development and association with allergy symptoms

For allergy development, clearly the direct contact to allergens and allergen sources is crucial. This holds also true for IgE against CCD. Grass pollen inhalation and insect stings are considered to be the most probable sensitization routes since in the grass pollen season as well as in the situation following a hymenoptera sting, it has been shown that the CCD-specific serum IgE concentration increases significantly.^{11,36,37} Additionally, triggers from the environment are known to contribute to induction and/or amplification of IgE responses including virus infections,³⁸ air pollution³⁹ or toxins.⁴⁰ Noteworthy, many plants well-known as sources for food allergens contain glycan-binding proteins (lectins). These are highly specific for distinct carbohydrate structures. Most lectins consist of more than one subunit, each able to bind to the respective specific glycan epitope. This multivalency can lead to cross-linking of glycoproteins on cell surfaces, such as receptor-bound IgE on mast cells and basophils, resulting in cell activation, degranulation, release of histamine and Th2 cytokines IL-4 and IL-13.^{41,42} High sensitization rates have been observed by consumption of insufficiently boiled white or red beans (*Phaseolus vulgaris*)⁴² and in workers in coffee industries due to contamination of the coffee

beans with Castor beans (*Ricinus communis*).⁴³ A significant factor for boosting IgE is the consumption of alcohol, as several studies documented an increased anti-CCD-IgE concentration in sera of alcoholics.^{44–46} The risk of CCD sensitization tended to increase with the consumed dose of alcohol. However, it has been described that these “alcohol-boosted” IgE against CCD exhibit a low grade of biologic activity *in vivo* (skin prick test negativity, no clinically relevant allergy symptoms).⁴⁵ The influence of alcohol on mucosa permeability and thus barrier integrity is a possible explanation for increased CCD sensitization of alcoholics. It is known that major triggers for IgE development are parasite infections, especially parasitic worms like helminths.^{47–49} Helminth-induced IgE antibodies are not only directed against worm antigens, but are also cross-reactive with known (food) allergens as shown for IgE to peanut allergens in Ghanaian school children.⁴⁷ Helminth antigens and common allergens have similar glycan structures, which are responsible for this IgE cross-reactivity (Fig. 3 and 5). Of note, despite the high concentration of specific IgE to peanut allergens, these school children develop no allergy symptoms upon exposure to peanut. Recently, it has been shown that immunization of rabbits with *Schistosoma mansoni* egg antigens resulted in induction of glycan-specific antibodies with high cross-reactivity to the latex allergen Hev b 7, as well as several food allergens.⁵⁰

6 Immunology of anti-glycan IgE associated with allergy or tolerance

Frequent contact with normally harmless proteins from food or the environment is generally associated with tolerance rather than with allergic reactions. However, during the development of allergy and specific IgE antibodies, these tolerance mechanisms are disturbed in some individuals.^{51–54} Even after the induction of specific IgE, some individuals do not develop allergy.⁴⁷ The underlying mechanisms leading to tolerance against these IgE-binding structures are still elusive. Crucial questions are: What is the difference between glycans, which induce IgE-mediated clinical symptoms (a-Gal) and glycans, which do not (classical CCDs)? Is the glycan structure essential, or does tolerance depend mainly on other (host-derived) factors? Or is a certain interaction necessary in order to develop allergy and clinical symptoms? Induction of tolerance can be observed during allergen-specific immunotherapy, or naturally in bee keepers, often in contact to bee venom, or individuals chronically infested with parasitic worms. Several mechanisms have been proposed to be involved in inhibition of clinical allergic symptoms, such as the induction of allergen-specific blocking IgG (IgG₄) antibodies^{55,56} or induction of inhibitory anti-FcγRIIb antibodies.^{57,58} The latter have been shown to inhibit mast cell degranulation by co-crosslinking FcεRI and FcγRIIb on the cell surface. Moreover, especially in the context of anti-glycan-IgE, the lack of clinical symptoms is explained by inefficient activation of FcεRI-expressing effector cells due to inefficient cross-linking of FcεRI-bound IgE on their surface. It has been proposed that the ratio of specific vs. total IgE is a marker for the manifestation of clinical allergy symptoms.⁵⁹ In order to activate basophils and mast cells (MC), a certain amount of receptor-bound, allergen-specific IgE has to be present on the cell surface. A high ratio results from either high concentrations of specific IgE or low concentrations of total serum

IgE and may be considered as a risk factor for IgE-mediated cell activation.⁵⁹ Concerning the clinical relevance of anti-CCD or anti-glycan IgE, especially the IgE affinity has been a major point of discussion. The affinity of anti-CCD IgE in the past was considered as generally low with a high degree of cross-reactivity. However, this is not always true. Very high anti-CCD IgE affinities of around 10^{-10} mol/L for MMF- and MMXF-transferrin, have been observed.³⁷ A possible reason for this discrepancy in observations are the two routes by which IgE antibodies are generated by isotype switch of a naïve B cell. There are studies pointing to the development of high-affinity (anti-CCD) IgE by a process of “indirect class switching” from IgM *via* IgG, including access to germinal centers and affinity maturation, to IgE (Fig. 4A).^{60,61} According to this hypothesis, low-affinity IgE would be derived from B cells via direct IgM – IgE class switch. Thus, the mere existence of IgG against a certain allergen has to be considered at least as a risk factor for allergy, because an IgG⁺ B cell is able to switch to IgE in a Th2 environment. It has been shown that this isotype switch happens preferably in IgG₁⁺ B cells.⁶² These allergen-specific IgG⁺ memory B cells resemble a constant reservoir of possible IgE-switching cells supporting the allergic disease. Taken together, high affinity IgE is predominantly derived from indirect class switch *via* IgG and IgG affinity maturation and may cause clinically relevant allergic reactions in patients. Concerning the possible induction of allergy symptoms by glycans, combined structural and functional aspects have to be taken into account: First, glycosylation of allergens is not uniform. A major proportion of a natural allergen may contain only one glycosylation site, thus cannot be cross-linked by anti-CCD antibodies. This is the case for the CCD-diagnostic molecule bromelain, which contains only one N-glycosylation site.¹⁶ Second, the steric localization of carbohydrates on allergens may not be suitable for efficient IgE binding and/or cross-linking. That means either the glycan on these allergens is sterically not accessible or the second glycan crucial for IgE cross-linking is not accessible for the neighboring cell-surface-receptor-bound IgE on the immune effector cell (MC, basophils). It has to be considered that allergen-specific B cells are selected *via* B cell-receptor recognition of peptides (and eventually attached carbohydrates) bound to MHC molecules.⁶³ In this way, specific IgE responses are also generated against monovalent allergens (e.g. CCD structures in the case where an allergen only contains one glycosylation site like bromelain). However, monovalent allergens are intrinsically not able to cross-link IgE and to efficiently activate effector immune cells. Thus, these allergens are IgE-binding but not cross-linking and therefore do not cause allergy symptoms. This phenomenon is also called “low grade of biological activity”.

7 Interaction of glycans with human carbohydrate receptors and impact on allergic inflammation - Functional aspects of glycans

Carbohydrate-specific receptors of different types and their interaction with glycans have been thoroughly characterized in molecular biology (Fig. 6).^{64–67} It is known that some glycosylated allergens interact with carbohydrate receptors on DCs, e.g. Fel d 1 to the mannose receptor (MR)⁶⁸ and Ara h 1 to DC-SIGN, this way inducing a pro-allergic Th2 immune response.⁷ On dendritic cells, there are two classes of C-type

lectin receptors: the mannose receptor (MR) and the DC-SIGN receptor.^{6,65,69} Although these receptors are both binding to carbohydrates, their specificity is fundamentally different. The MR binds to Man, GlcNAc and Fuc whereas the DC-SIGN receptor is binding to the pro-inflammatory and complement-activating high mannose glycan motif.^{5,70–72} It has been published that glycosylated allergens that either bind to the MR or DC-SIGN induce different pro-allergic and anti-allergic immunological mechanisms as has been observed for single house dust mite and peanut allergens. However, it is a matter of debate whether the allergen interaction with a specific carbohydrate receptor on DCs is promoting allergy.⁷⁰ Moreover, glycan-binding toll-like receptors (TLR) 2, 4 and 6 are present on various types of immune cells, e.g. DC, macrophages and monocytes. While allergens bind to MR or DC-SIGN receptors, TLR-mediated signaling may have an additional impact on the characteristics of the allergen-induced inflammation. Often, the function of these carbohydrate-specific receptors is not understood in detail, e.g. which exact glycan structure is able to activate a certain carbohydrate receptor. An overview of human receptors and their so far identified glycan ligands is provided in Figure 6.

One example for potential anti-inflammatory property of a glycan structure is the negatively charged carbohydrate sialic acid. It has been found that sialylated antibodies possess anti-inflammatory properties.^{54,73,74} IgG antibodies lacking sialic acid and varying in galactosylation in the antennae of N-glycans are enhanced in autoimmune processes and drive the disease due to their interaction with pro-inflammatory, activating Fcγ receptors.^{73,74} Furthermore, the proliferation of NK- and T-cells is inhibited by a receptor specific for sialylated glycans (SIGLEC). SIGLEC-2 for example, binds to the sialylated N-acetyl lactosamine (LacNAc) motif where interaction with this disaccharide is inhibited by sialic acid.⁷⁵ Carbohydrate patterns on proteins are also able to induce differential cellular responses depending on the specific glycan receptor. For the non-human sialic acid, the N-glycolyl neuraminic acid (Neu5Gc), the antigenic and IgE-inducing properties are presently a matter of debate.⁷⁶ Although it has been shown that the monosaccharide Neu5Gc linked to a hydrocarbon spacer is not IgE-reactive,²⁹ it is published that antibody responses against Neu5Gc exist.⁷⁷ A specific feature for Neu5Gc as a non-human carbohydrate is that it is taken up from nutrition and readily incorporated into human glycoproteins.^{78,79} The consequences of this metabolic process are not clear. On the one hand, by this incorporation and constant exposition to the human immune system, tolerance against Neu5Gc may be induced. However, it has been shown that a substantial percentage of people possess antibodies against Neu5Gc and that these antibodies are inducing an inflammatory environment in the vicinity of cells which contain Neu5Gc.^{77,80}

Lewis (Le) antigens derived from non-human sources like plant allergens or parasites are similar or identical to human glycans and interact with the human immune system. These carbohydrates have exactly the same structure as human glycans, but are, for example, linked to a foreign protein (Fig. 2 and 4). Some identical human glycan structures are highly active signaling molecules on the surface of immune cells. Therefore, it is reasonable to conclude that these glycans which are “similar to human glycans but from non-human sources” also have a

significant impact on cell signaling processes and the immune system as well. One well-known Le^x-specific carbohydrate receptor is selectin in its isoform CD62E which is part of the process of so-called “leukocyte rolling” in inflammation. It may be speculated that also other Le^x-containing allergens/proteins are able to interact with CD62E-expressing cells which may direct allergens to cells with an inflammatory environment. Lewis antigens have very distinct and specific receptors on human cells (Fig. 5 and 6). Le^x for example is binding to carbohydrate receptors MR and DC-SIGN on the surface of antigen-presenting cells like DCs. As a consequence, allergen-relevant Th2 cytokines and IL-10 are induced.⁴ Addressing the parasite infection model for the induction of allergic diseases, it has been described that several schistosomal glycoproteins are mimicking human glycosylation patterns. Among these are the Lewis-type glycans and also related variations of LDN glycans (Fig. 3 and 5). It may be speculated that some of the glycans from parasites as well as glycans on allergens which are similar to human glycans, interact with the human receptors in the same or a similar way as the human glycans. During some of these interactions, an immunological bias towards Th2 is induced.⁸¹

8 Allergy, Th2 inflammation and parasite glycans as a model for the development of anti-glycan IgE

Allergy-related Th2 responses including development of IgE antibodies is a hallmark of an infection with parasitic worms.^{82,83} Acute helminth infection can lead to symptoms similar to allergic inflammation including urticarial wheals and itching at the site of infection, coughing, fever, headache, skin rash, abdominal pain and a general feeling of illness.⁸⁴ In contrast, during the chronic infection phase, the human immune system shifts from a Th2-driven inflammation, which is considered as the defense mechanism against parasites, to an anti-inflammatory, so called regulated or modified Th2 immune response.⁸³ Carbohydrates play an important role in immune priming process.^{85,86} This involves production of anti-inflammatory IL-10, induction of regulatory cells, such as T_{regs} and B_{regs} and alternatively activated macrophages (AAM).⁸⁷ While the Th2-associated IgE production is described to be effective to repel intestinal parasites, it is not known why the same immune response develops against parasitic worms which migrate through tissues and live in the blood vessels, such as *Schistosoma mansoni*.⁸⁸

8.1 Development and cross-reactivity of anti-glycan IgE during infection with *Schistosoma mansoni*

During the course of the infection with *S. mansoni*, a significant proportion of anti-parasite antibodies are IgE antibodies directed against parasite glycans, which partly have the same structure as classical CCD in a variety of plant allergen sources (Fig. 4 and 5). However, this anti-glycan IgE does not lead to killing of the parasite, and the immunological function of this anti-glycan IgE is not known – mirroring the situation for anti-CCD IgE in allergy. Like in allergy research, there is also an ongoing discussion about the characteristics and effects of anti-glycan IgE induced

by the parasite infection. Interestingly, African children possess a high concentration of peanut-cross-reactive IgE, most likely induced by parasite infection.⁴⁷ However, as known from anti-CCD-IgE in allergy patients in Western Europe, this glycan-specific IgE does not cause any clinical symptoms.⁴⁷ In the infection process of the helminth *Schistosoma mansoni*, IgE receptors on eosinophils and B cells (or soluble in patients' serum), respectively, are associated with protection against reinfection.^{82,89–91} In contrast, susceptibility to reinfection has been associated with IgG₄, which may serve as a blocking antibody, inhibiting the action of IgE.^{82,92–94} Three major glycoproteins which are derived from schistosomal eggs are known as major components of the immune modulation (towards Th2) and IgE induction in the human host. The secreted egg proteins IPSE/alpha-1 (*IL-4-inducing Principle from Schistosoma mansoni Eggs*) and Omega-1 have pronounced immunomodulatory functions.^{95,96} IPSE/α-1 was shown to bind to receptor-bound IgE thus triggering IL-4 release from human basophils independent of the specificity of the receptor-bound IgE on these cells. Omega-1 induces a Th2 polarization by conditioning human DCs depending on its ribonuclease function.⁹⁵ While IPSE/α-1 functions independently of its glycosylation, omega-1 activity crucially depends on its carbohydrates which mediate its uptake into DCs via mannose receptor in DCs.^{95,97} The glycans which mediate this uptake are difucosylated N-glycan core structures containing Le^x in the antennae (Fig. 4 and 5). Interestingly, although involved in Th2 and subsequent IgE induction, in infected patients no IgE has been detected which is directed against IPSE/α-1 and Omega-1. In contrast, a pronounced IgE response was detected against a third, non-secreted egg protein, Kappa-5,⁹⁸ and here against the carbohydrates. This difference becomes intriguing when the difference in the glycosylation patterns is taken into account: Kappa-5 contains the non-human carbohydrates xylose and α-(1,3)-linked fucose in the chitobiose core (Fig. 5).⁹⁸ This structure mirrors exactly the classical CCD structure of MMXF on allergens, such as the “prototypic” CCD-containing protein HRP. Most likely the higher content of the described foreign, immunogenic glycans of Kappa-5 leads to the observed IgE response which is induced and supported by the other schistosomal egg proteins IPSE/alpha-1 (by induction of IL-4 release) and omega-1 (by induction of Th2).

9 Summary and Outlook: Perspectives of allergy diagnostics, pathomechanisms and treatment options in the light of pro-allergenic glycans and anti-glycan IgE

Considering the described clinically relevant cases of meat/innard allergy, anti-glycan IgE cannot be ignored in allergy diagnostics and mechanistic allergy research. In terms of allergy diagnostics, the nature of the carbohydrate-containing allergen should be taken into account. There are two major aspects of the clinically relevant allergic reaction: First, the allergen site with the protein structure and potentially attached glycans resulting in no, mono- or multivalent IgE epitopes suitable for IgE cross-linking. In order to induce a clinically relevant allergic reaction, an allergen must have properties to cross-link IgE. This process activates MC and basophils and leads to secretion of pro-inflammatory mediators. There are two ways to cross-link receptor-bound IgE on the surface of immune cells for an allergen:

1 Either it must have multiple binding sites for IgE or it must be
2 oligomerized/polymerized to present the same epitope multiple times and sterically
3 optimal for IgE cross-linking and receptor dimerization. Other features like the
4 enzymatic activity are also important for some allergens. The second important point
5 for allergy development is the “host” side, i.e. the human individual. Crucial factors
6 are cellular properties like FcεR expression on MC and basophils, the glycan
7 receptor profile and the priming and activation of the immune system in the direction
8 of a predominant Th2 response. An important aspect of immunological tolerance is
9 the concentration of allergen-specific antibodies of isotypes other than IgE.
10 Especially, the IgG subclass IgG₄ has been shown in many studies to be a blocking,
11 tolerance-inducing, thus “anti-allergenic” antibody (Fig. 4D).^{55,56,99} The induction of
12 tolerance and blocking antibodies is a major goal of the allergen-specific
13 immunotherapy. Another important aspect of allergic reactions is the ratio of
14 allergen-specific vs. total serum IgE. A high ratio results in a higher activation of
15 immune cells with specific IgE by an allergen and is considered as a risk factor for
16 allergic symptoms.⁵⁹ In terms of allergy diagnostics, it has to be considered that the
17 widely used single- and multiplex IgE detection systems are not equally suitable to
18 detect A) an IgE reaction to every analyzed allergen, especially if recombinant
19 allergens are used, due to structural differences like the missing or altered allergen
20 glycosylation, disulfide bonds or other, potentially also dynamic, protein
21 modifications. And B) thus, the determined allergen-specific IgE concentration may
22 not resemble the true IgE-binding potential of the allergen. The consequence is that
23 the commercial IgE detection systems may be missing relevant natural allergenic
24 glycans and also may not be sensitive enough to detect clinically relevant IgE
25 against carbohydrates. The difference in IgE reactivity and the necessity to use
26 natural allergens or allergens with a glycosylation pattern similar to the natural
27 glycosylation has to be determined for each allergen as not all allergens are
28 glycosylated and the glycosylation may not be equally important for IgE binding to
29 every allergen. Chronic infections with *S. mansoni* elicit high IgE serum levels with
30 IgE directed against cross-reactive carbohydrate determinants, present on
31 schistosomal antigens as well as on allergens. Strikingly, it is known that helminth
32 infection protects against allergy despite high allergen-specific IgE.^{47,87} Thus, it is a
33 matter of debate if anti-CCD IgE may be even allergy-protective by inhibition of
34 allergic symptoms. In this regard, induction of anti-CCD antibodies might be
35 interesting as a novel immunotherapy concept for the treatment of allergic
36 diseases.⁵⁰

References

1. Aalberse RC, Akkerdaas J, Van Ree R. Cross-reactivity of IgE antibodies to allergens. *Allergy* 2001;56:478–490.
2. van Ree R. beta (1,2)-Xylose and alpha (1,3)-Fucose Residues Have a Strong Contribution in IgE Binding to Plant Glycoallergens. *J Biol Chem* 2000;275:11451–8.
3. Commins SP. Carbohydrates as Allergens. *Curr Allergy Asthma Rep* [Internet] 2015 [cited 2015 Jul 27];15. Available from: <http://link.springer.com/10.1007/s11882-014-0492-y>
4. Hsu S-C, Tsai T-H, Kawasaki H, Chen C-H, Plunkett B, Lee RT, et al. Antigen coupled with Lewis-x trisaccharides elicits potent immune responses in mice. *J Allergy Clin Immunol* 2007;119:1522–8.
5. Salazar F, Sewell HF, Shakib F, Ghaemmamghami AM. The role of lectins in allergic sensitization and allergic disease. *J Allergy Clin Immunol* 2013;132:27–36.
6. Van Vliet SJ, García-Vallejo JJ, van Kooyk Y. Dendritic cells and C-type lectin receptors: coupling innate to adaptive immune responses. *Immunol Cell Biol* 2008;86:580–587.
7. Shreffler WG, Castro RR, Kucuk ZY, Charlop-Powers Z, Grishina G, Yoo S, et al. The major glycoprotein allergen from *Arachis hypogaea*, Ara h 1, is a ligand of dendritic cell-specific ICAM-grabbing nonintegrin and acts as a Th2 adjuvant in vitro. *J Immunol Baltim Md 1950* 2006;177:3677–85.
8. Kolarich D, Altmann F. N-Glycan Analysis by Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry of Electrophoretically Separated Nonmammalian Proteins: Application to Peanut Allergen Ara h 1 and Olive Pollen Allergen Ole e 1. *Anal Biochem* 2000;285:64–75.
9. Harn DA, McDonald J, Atochina O, Da'dara AA. Modulation of host immune responses by helminth glycans. *Immunol Rev* 2009;230:247–57.
10. Brehler R, Grundmann S, Stöcker B. Cross-reacting carbohydrate determinants and hymenoptera venom allergy: *Curr Opin Allergy Clin Immunol* 2013;13:360–4.
11. Jappe U, Petersen A, Raulf-Heimsoth M. Immediate-type allergic reactions and cross-reactive carbohydrate determinants (CCD). *Allergo J Int* 2013;22.
12. Holzweber F, Svehla E, Fellner W, Dalik T, Stubler S, Hemmer W, et al. Inhibition of IgE binding to cross-reactive carbohydrate determinants enhances diagnostic selectivity. *Allergy* 2013;68:1269–77.
13. Jappe U, Raulf-Heimsoth M, Hoffmann M, Burow G, Hübsch-Müller C, Enk A. In vitro hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. *Allergy* 2006;61:1220–9.
14. Worm M, Jappe U, Kleine-Tebbe J, Schäfer C, Reese I, Saloga J, et al. Food allergies resulting from immunological cross-reactivity with inhalant allergens. *Allergo J Int* 2014;23:1–16.
15. Foetisch K, Westphal S, Lauer I, Retzek M, Altmann F, Kolarich D, et al. Biological activity of IgE specific for cross-reactive carbohydrate determinants. *J Allergy Clin Immunol* 2003;111:889–96.
16. Malandain H. IgE-reactive carbohydrate epitopes--classification, cross-reactivity, and clinical impact. *Eur Ann Allergy Clin Immunol* 2005;37:122–8.
17. Mari A, Ooievaar-de Heer P, Scala E, Giani M, Pirrotta L, Zuidmeer L, et al. Evaluation by double-blind placebo-controlled oral challenge of the clinical relevance of IgE antibodies against plant glycans: Oral challenge with transgenic glycosylated proteins. *Allergy* 2008;63:891–6.
18. van der Veen MJ, van Ree R, Aalberse RC, Akkerdaas J, Koppelman SJ, Jansen HM, et al. Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. *J Allergy Clin Immunol* 1997;100:327–334.
19. Eberlein B, Krischan L, Darsow U, Ollert M, Ring J. Double positivity to bee and wasp venom: Improved diagnostic procedure by recombinant allergen-based IgE testing and basophil activation test including data about cross-reactive carbohydrate determinants. *J Allergy Clin Immunol* 2012;130:155–61.

20. Westphal S, Kolarich D, Foetisch K, Lauer I, Altmann F, Conti A, et al. Molecular characterization and allergenic activity of Lyc e 2 (β -fructofuranosidase), a glycosylated allergen of tomato. *Eur J Biochem* 2003;270:1327–37.
21. Altmann F. The Role of Protein Glycosylation in Allergy. *Int Arch Allergy Immunol* 2007;142:99–115.
22. Commins SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD, et al. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose- α -1,3-galactose. *J Allergy Clin Immunol* 2009;123:426–433.e2.
23. Chung CH, Mirakhur B, Chan E, Le Q-T, Berlin J, Morse M, et al. Cetuximab-Induced Anaphylaxis and IgE Specific for Galactose- α -1,3-Galactose. *N Engl J Med* 2008;358:1109–17.
24. Rispens T, Derksen NIL, Commins SP, Platts-Mills TA, Aalberse RC. IgE Production to α -Gal Is Accompanied by Elevated Levels of Specific IgG1 Antibodies and Low Amounts of IgE to Blood Group B. *PLoS ONE* 2013;8:e55566.
25. Galili U, Shohet SB, Kobrin E, Stults CL, Macher BA. Man, apes, and Old World monkeys differ from other mammals in the expression of alpha-galactosyl epitopes on nucleated cells. *J Biol Chem* 1988;263:17755–62.
26. Ayoub D, Jabs W, Resemann A, Evers W, Evans C, Main L, et al. Correct primary structure assessment and extensive glyco-profiling of cetuximab by a combination of intact, middle-up, middle-down and bottom-up ESI and MALDI mass spectrometry techniques. *mAbs* 2013;5:699–710.
27. Qian J, Liu T, Yang L, Daus A, Crowley R, Zhou Q. Structural characterization of N-linked oligosaccharides on monoclonal antibody cetuximab by the combination of orthogonal matrix-assisted laser desorption/ionization hybrid quadrupole–quadrupole time-of-flight tandem mass spectrometry and sequential enzymatic digestion. *Anal Biochem* 2007;364:8–18.
28. Stadlmann J, Pabst M, Kolarich D, Kunert R, Altmann F. Analysis of immunoglobulin glycosylation by LC-ESI-MS of glycopeptides and oligosaccharides. *PROTEOMICS* 2008;8:2858–71.
29. Apostolovic D, Tran T a. T, Sánchez-Vidaurre S, Cirkovic Velickovic T, Starkhammar M, Hamsten C, et al. Red meat allergic patients have a selective IgE response to the α -Gal glycan. *Allergy* 2015;n/a-n/a.
30. Jappe U. Update on meat allergy. alpha-Gal: a new epitope, a new entity? *Hautarzt* 2012;63:299–306.
31. Commins SP, James HR, Kelly LA, Pochan SL, Workman LJ, Perzanowski MS, et al. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose- α -1,3-galactose. *J Allergy Clin Immunol* 2011;127:1286–1293.e6.
32. Sundaram S, Matathia A, Qian J, Zhang J, Hsieh M-C, Liu T, et al. An innovative approach for the characterization of the isoforms of a monoclonal antibody product. *mAbs* 2011;3:505–12.
33. Rispens T, Heer PO-D, Vermeulen E, Schuurman J, Kolfschoten M van der N, Aalberse RC. Human IgG4 Binds to IgG4 and Conformationally Altered IgG1 via Fc-Fc Interactions. *J Immunol* 2009;182:4275–81.
34. Mullins RJ, James H, Platts-Mills TAE, Commins S. Relationship between red meat allergy and sensitization to gelatin and galactose- α -1,3-galactose. *J Allergy Clin Immunol* 2012;129:1334–1342.e1.
35. Roncati L, Barbolini G, Scacchetti AT, Busani S, Maiorana A. Unexpected death: Anaphylactic intraoperative death due to Thymoglobulin carbohydrate excipient. *Forensic Sci Int* 2013;228:e28–32.

36. Bencurova M. Specificity of IgG and IgE antibodies against plant and insect glycoprotein glycans determined with artificial glycoforms of human transferrin. *Glycobiology* 2004;14:457–66.
37. Jin C, Hantusch B, Hemmer W, Stadlmann J, Altmann F. Affinity of IgE and IgG against cross-reactive carbohydrate determinants on plant and insect glycoproteins. *J Allergy Clin Immunol* 2008;121:185–190.e2.
38. Becker Y. Respiratory syncytial virus (RSV) evades the human adaptive immune system by skewing the Th1/Th2 cytokine balance toward increased levels of Th2 cytokines and IgE, markers of allergy--a review. *Virus Genes* 2006;33:235–52.
39. Brandt EB, Kovacic MB, Lee GB, Gibson AM, Acciani TH, Le Cras TD, et al. Diesel exhaust particle induction of IL-17A contributes to severe asthma. *J Allergy Clin Immunol* 2013;132:1194–1204.e2.
40. Behrendt H, Alessandrini F, Buters J, Krämer U, Koren H, Ring J. Environmental pollution and allergy: historical aspects. *Chem Immunol Allergy* 2014;100:268–77.
41. Haas H, Falcone FH, Schramm G, Haisch K, Gibbs BF, Klaucke J, et al. Dietary lectins can induce in vitro release of IL-4 and IL-13 from human basophils. *Eur J Immunol* 1999;29:918–27.
42. Kumar S, Verma AK, Sharma A, Kumar D, Tripathi A, Chaudhari BP, et al. Phytohemagglutinins augment red kidney bean (*Phaseolus vulgaris* L.) induced allergic manifestations. *J Proteomics* 2013;93:50–64.
43. Romano C, Sulotto F, Piolatto G, Ciacco C, Capellaro E, Falagiani P, et al. Factors related to the development of sensitization to green coffee and castor bean allergens among coffee workers. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 1995;25:643–50.
44. Gonzalez-Quintela A, Garrido M, Gude F, Campos J, Linneberg A, Lojo S, et al. Sensitization to cross-reactive carbohydrate determinants in relation to alcohol consumption. *Clin Exp Allergy* 2008;38:152–60.
45. Linneberg A, Fenger RV, Husemoen L-LN, Vidal C, Vizcaino L, Gonzalez-Quintela A. Immunoglobulin E Sensitization to Cross-Reactive Carbohydrate Determinants: Epidemiological Study of Clinical Relevance and Role of Alcohol Consumption. *Int Arch Allergy Immunol* 2010;153:86–94.
46. Vidal C, Vizcaino L, Díaz-Peromingo JA, Garrido M, Gomez-Rial J, Linneberg A, et al. Immunoglobulin-E Reactivity to a Glycosylated Food Allergen (Peanuts) Due to Interference With Cross-Reactive Carbohydrate Determinants in Heavy Drinkers. *Alcohol Clin Exp Res* 2009;33:1322–8.
47. Amoah AS, Obeng BB, Larbi IA, Versteeg SA, Aryeetey Y, Akkerdaas JH, et al. Peanut-specific IgE antibodies in asymptomatic Ghanaian children possibly caused by carbohydrate determinant cross-reactivity. *J Allergy Clin Immunol* 2013;132:639–47.
48. Maizels RM, Hewitson JP. Immune Recognition of Parasite Glycans. *Anticarbhydrate Antibodies* 2012;161–180.
49. Meevissen MHJ, Driessen NN, Smits HH, Versteegh R, van Vliet SJ, van Kooyk Y, et al. Specific glycan elements determine differential binding of individual egg glycoproteins of the human parasite *Schistosoma mansoni* by host C-type lectin receptors. *Int J Parasitol* 2012;42:269–77.
50. Doenhoff MJ, El-Faham M, Liddell S, Fuller HR, Stanley RG, Schramm G, et al. Cross-Reactivity between *Schistosoma mansoni* Antigens and the Latex Allergen Hev b 7: Putative Implication of Cross-Reactive Carbohydrate Determinants (CCDs). *PLOS ONE* 2016;11:e0159542.
51. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: Multiple suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol* 2014;133:621–31.
52. Lu M, Varley AW, Munford RS. Persistently Active Microbial Molecules Prolong Innate Immune Tolerance In Vivo. *PLoS Pathog* 2013;9:e1003339.

53. Medzhitov R, Schneider DS, Soares MP. Disease tolerance as a defense strategy. *Science* 2012;335:936–941.
54. Oefner CM, Winkler A, Hess C, Lorenz AK, Holecska V, Huxdorf M, et al. Tolerance induction with T cell–dependent protein antigens induces regulatory sialylated IgGs. *J Allergy Clin Immunol* 2012;129:1647–1655.e13.
55. James LK, Till SJ. Potential Mechanisms for IgG4 Inhibition of Immediate Hypersensitivity Reactions. *Curr Allergy Asthma Rep* 2016;16:23.
56. Santos AF, James LK, Bahnson HT, Shamji MH, Couto-Francisco NC, Islam S, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. *J Allergy Clin Immunol* 2015;135:1249–56.
57. MacGlashan Jr. D, Hamilton RG. Parameters determining the efficacy of CD32 to inhibit activation of FcεRI in human basophils. *J Allergy Clin Immunol* 2016;137:1256–1258.e11.
58. Uermösi C, Zabel F, Manolova V, Bauer M, Beerli RR, Senti G, et al. IgG-mediated down-regulation of IgE bound to mast cells: a potential novel mechanism of allergen-specific desensitization. *Allergy* 2014;69:338–47.
59. Kleine-Tebbe J, Jakob T. Molecular allergy diagnostics using IgE singleplex determinations: methodological and practical considerations for use in clinical routine: Part 18 of the Series Molecular Allergology. *Allergo J Int* 2015;24:185.
60. Aalberse RC, Platts-Mills TAE. How do we avoid developing allergy: Modifications of the TH2 response from a B-cell perspective. *J Allergy Clin Immunol* 2004;113:983–6.
61. Dullaers M, De Bruyne R, Ramadani F, Gould HJ, Gevaert P, Lambrecht BN. The who, where, and when of IgE in allergic airway disease. *J Allergy Clin Immunol* 2012;129:635–45.
62. Looney TJ, Lee J-Y, Roskin KM, Hoh RA, King J, Glanville J, et al. Human B-cell isotype switching origins of IgE. *J Allergy Clin Immunol* 2016;137:579–586.e7.
63. Wolfert MA, Boons G-J. Adaptive immune activation: glycosylation does matter. *Nat Chem Biol* 2013;9:776.
64. Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. *Nature* 2000;406:782–7.
65. Geijtenbeek TBH, Gringhuis SI. Signalling through C-type lectin receptors: shaping immune responses. *Nat Rev Immunol* 2009;9:465–79.
66. Homann A, Timm M, Seibel J. Chemo-enzymatic synthesis and in vitro cytokine profiling of tailor-made oligofructosides. *BMC Biotechnol* 2012;12:90.
67. Homann A, Seibel J. Chemo-enzymatic synthesis and functional analysis of natural and modified glycostructures. *Nat Prod Rep* 2009;26:1555–71.
68. Emara M, Royer P-J, Abbas Z, Sewell HF, Mohamed GG, Singh S, et al. Recognition of the Major Cat Allergen Fel d 1 through the Cysteine-rich Domain of the Mannose Receptor Determines Its Allergenicity. *J Biol Chem* 2011;286:13033–40.
69. Geijtenbeek TBH, Engering A, Kooyk Y van. DC-SIGN, a C-type lectin on dendritic cells that unveils many aspects of dendritic cell biology. *J Leukoc Biol* 2002;71:921–31.
70. Al-Ghouleh A, Johal R, Sharquie IK, Emara M, Harrington H, Shakib F, et al. The Glycosylation Pattern of Common Allergens: The Recognition and Uptake of Der p 1 by Epithelial and Dendritic Cells Is Carbohydrate Dependent. *PLoS ONE* 2012;7:e33929.
71. Royer P-J, Emara M, Yang C, Al-Ghouleh A, Tighe P, Jones N, et al. The Mannose Receptor Mediates the Uptake of Diverse Native Allergens by Dendritic Cells and Determines Allergen-Induced T Cell Polarization through Modulation of IDO Activity. *J Immunol* 2010;185:1522–31.
72. Sharquie IK, Al-Ghouleh A, Fitton P, Clark MR, Armour KL, Sewell HF, et al. An investigation into IgE-facilitated allergen recognition and presentation by human dendritic cells. *BMC Immunol* 2013;14:54.
73. Collin M, Ehlers M. The carbohydrate switch between pathogenic and immunosuppressive antigen-specific antibodies. *Exp Dermatol* 2013;22:511–4.

74. Karsten CM, Pandey MK, Figge J, Kilchenstein R, Taylor PR, Rosas M, et al. Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcγRIIB and dectin-1. *Nat Med* 2012;18:1401–6.
75. Bochner BS, Zimmermann N. Role of siglecs and related glycan-binding proteins in immune responses and immunoregulation. *J Allergy Clin Immunol* 2015;135:598–608.
76. Ghaderi D, Taylor RE, Padler-Karavani V, Diaz S, Varki A. Implications of the presence of N-glycolylneuraminic acid in recombinant therapeutic glycoproteins. *Nat Biotechnol* 2010;28:863–7.
77. Pearce OMT, Läubli H. Sialic acids in cancer biology and immunity. *Glycobiology* 2016;26:111–28.
78. Homann A, Qamar R, Serim S, Dersch P, Seibel J. Bioorthogonal metabolic glycoengineering of human larynx carcinoma (HEp-2) cells targeting sialic acid. *Beilstein J Org Chem* [Internet] 2010 [cited 2016 Oct 31];6. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2874417/>
79. Tangvoranuntakul P, Gagneux P, Diaz S, Bardor M, Varki N, Varki A, et al. Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid. *Proc Natl Acad Sci* 2003;100:12045–50.
80. Samraj AN, Pearce OMT, Läubli H, Crittenden AN, Bergfeld AK, Banda K, et al. A red meat-derived glycan promotes inflammation and cancer progression. *Proc Natl Acad Sci* 2015;112:542–7.
81. Die I van, Vliet SJ van, Nyame AK, Cummings RD, Bank CMC, Appelmelk B, et al. The dendritic cell-specific C-type lectin DC-SIGN is a receptor for *Schistosoma mansoni* egg antigens and recognizes the glycan antigen Lewis x. *Glycobiology* 2003;13:471–8.
82. Colley DG, Secor WE. Immunology of human schistosomiasis. *Parasite Immunol* 2014;36:347–57.
83. Schramm G, Haas H. Th2 immune response against *Schistosoma mansoni* infection. *Microbes Infect* 2010;12:881–8.
84. Gryseels B. Schistosomiasis. *Infect Dis Clin North Am* 2012;26:383–97.
85. Okano M, Satoskar AR, Nishizaki K, Abe M, Harn DA. Induction of Th2 responses and IgE is largely due to carbohydrates functioning as adjuvants on *Schistosoma mansoni* egg antigens. *J Immunol Baltim Md* 1950 1999;163:6712–7.
86. Smit CH, Homann A, Hensbergen VP van, Schramm G, Haas H, Diepen A van, et al. Surface expression patterns of defined glycan antigens change during *Schistosoma mansoni* cercarial transformation and development of schistosomula. *Glycobiology* 2015;cwv066.
87. Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat Rev Immunol* 2003;3:733–44.
88. MacDonald AS, Araujo MI, Pearce EJ. Immunology of Parasitic Helminth Infections. *Infect Immun* 2002;70:427–33.
89. Ganley-Leal LM, Mwinzi PNM, Cetre-Sossah CB, Andove J, Hightower AW, Karanja DMS, et al. Higher Percentages of Circulating Mast Cell Precursors Correlate with Susceptibility to Reinfection with *Schistosoma Mansoni*. *Am J Trop Med Hyg* 2006;75:1053–7.
90. Mwinzi PNM, Ganley-Leal L, Black CL, Secor WE, Karanja DMS, Colley DG. Circulating CD23+ B Cell Subset Correlates with the Development of Resistance to *Schistosoma mansoni* Reinfection in Occupationally Exposed Adults Who Have Undergone Multiple Treatments. *J Infect Dis* 2009;199:272–9.
91. SoussiGounni A, Lamkhioued B, Ochiai K, Tanaka Y, Delaporte E, Capron A, et al. High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature* 1994;367:183–6.
92. Demeure CE, Rihet P, Abel L, Ouattara M, Bourgois A, Dessein AJ. Resistance to *Schistosoma mansoni* in Humans: Influence of the IgE/IgG4 Balance and IgG2 in Immunity to Reinfection after Chemotherapy. *J Infect Dis* 1993;168:1000–8.

- 1 93. Hagan P, Blumenthal UJ, Dunn D, Simpson AJG, Wilkins HA. Human IgE, IgG4 and resistance
2 to reinfection with *Schistosoma haematobium*. *Nature* 1991;349:243–5.
- 3 94. Zhang Z, Wu H, Chen S, Hu L, Xie Z, Qiu Y, et al. Association between IgE antibody against
4 soluble egg antigen and resistance to reinfection with *Schistosoma japonicum*. *Trans R Soc*
5 *Trop Med Hyg* 1997;91:606–8.
- 6 95. Everts B, Hussaarts L, Driessen NN, Meevissen MHJ, Schramm G, Ham AJ van der, et al.
7 Schistosome-derived omega-1 drives Th2 polarization by suppressing protein synthesis
8 following internalization by the mannose receptor. *J Exp Med* 2012;209:1753–67.
- 9 96. Meyer NH, Mayerhofer H, Tripsianes K, Blindow S, Barths D, Mewes A, et al. A Crystallin Fold
10 in the Interleukin-4-inducing Principle of *Schistosoma mansoni* Eggs (IPSE/ α -1) Mediates IgE
11 Binding for Antigen-independent Basophil Activation. *J Biol Chem* 2015;290:22111–26.
- 12 97. Meevissen MHJ, Wuhrer M, Doenhoff MJ, Schramm G, Haas H, Deelder AM, et al. Structural
13 Characterization of Glycans on Omega-1, a Major *Schistosoma mansoni* Egg Glycoprotein
14 That Drives Th2 Responses. *J Proteome Res* 2010;9:2630–42.
- 15 98. Meevissen MHJ, Balog CIA, Koeleman CAM, Doenhoff MJ, Schramm G, Haas H, et al.
16 Targeted Glycoproteomic Analysis Reveals That Kappa-5 is a Major, Uniquely Glycosylated
17 Component of *Schistosoma mansoni* Egg Antigens. *Mol Cell Proteomics*
18 2011;10:M110.005710.
- 19 99. Subbarayal B, Schiller D, Möbs C, de Jong NW, Ebner C, Reider N, et al. Kinetics, cross-
20 reactivity, and specificity of Bet v 1-specific IgG4 antibodies induced by immunotherapy with
21 birch pollen. *Allergy* 2013;68:1377–86.
- 22 100. Schramm G, Hamilton JV, Balog CIA, Wuhrer M, Gronow A, Beckmann S, et al. Molecular
23 characterisation of kappa-5, a major antigenic glycoprotein from *Schistosoma mansoni* eggs.
24 *Mol Biochem Parasitol* 2009;166:4–14.
- 25 101. Wuhrer M, Balog CIA, Catalina MI, Jones FM, Schramm G, Haas H, et al. IPSE/ α -1, a
26 major secretory glycoprotein antigen from schistosome eggs, expresses the Lewis X motif on
27 core-difucosylated N-glycans. *FEBS J* 2006;273:2276–92.
- 28 102. Nyame AK, Pilcher JB, Tsang VCW, Cummings RD. *Schistosoma mansoni* Infection in Humans
29 and Primates Induces Cytolytic Antibodies to Surface LexDeterminants on Myeloid Cells. *Exp*
30 *Parasitol* 1996;82:191–200.
- 31 103. Srivatsan J, Smith DF, Cummings RD. The human blood fluke *Schistosoma mansoni*
32 synthesizes glycoproteins containing the Lewis X antigen. *J Biol Chem* 1992;267:20196–203.
- 33 104. Velupillai P, Harn DA. Oligosaccharide-specific induction of interleukin 10 production by
34 B220+ cells from schistosome-infected mice: a mechanism for regulation of CD4+ T-cell
35 subsets. *Proc Natl Acad Sci U S A* 1994;91:18–22.
- 36 105. Neeleman AP, van der Knaap WP, van den Eijnden DH. Identification and characterization of
37 a UDP-GalNAc:GlcNAc beta-R beta 1-->4-N-acetylgalactosaminyltransferase from cercariae
38 of the schistosome *Trichobilharzia ocellata*. Catalysis of a key step in the synthesis of N,N'-
39 diacetyllactosedi amino (lacdiNAc)-type glycans. *Glycobiology* 1994;4:641–51.
- 40 106. Nyame AK, Lewis FA, Doughty BL, Correa-Oliveira R, Cummings RD. Immunity to
41 schistosomiasis: glycans are potential antigenic targets for immune intervention. *Exp*
42 *Parasitol* 2003;104:1–13.
- 43 107. Nyame AK, Leppanen AM, DeBose-Boyd R, Cummings RD. Mice infected with *Schistosoma*
44 *mansoni* generate antibodies to LacdiNAc (GalNAc beta 1-->4GlcNAc) determinants.
45 *Glycobiology* 1999;9:1029–35.
- 46 108. Srivatsan J, Smith DF, Cummings RD. *Schistosoma mansoni* synthesizes novel biantennary
47 Asn-linked oligosaccharides containing terminal β -linked N-acetylgalactosamine.
48 *Glycobiology* 1992;2:445–52.
- 49 109. Van de Vijver KK, Deelder AM, Jacobs W, Van Marck EA, Hokke CH. LacdiNAc- and LacNAc-
50 containing glycans induce granulomas in an in vivo model for schistosome egg-induced
51 hepatic granuloma formation. *Glycobiology* 2006;16:237–43.

- 1 110. van den Berg TK, Honing H, Franke N, van Remoortere A, Schiphorst WECM, Liu FT, et al.
2 LacdiNAc-glycans constitute a parasite pattern for galectin-3-mediated immune recognition.
3 J Immunol 2004;173:1902.
- 4 111. van Vliet SJ, van Liempt E, Saeland E, Aarnoudse CA, Appelmelk B, Irimura T, et al.
5 Carbohydrate profiling reveals a distinctive role for the C-type lectin MGL in the recognition
6 of helminth parasites and tumor antigens by dendritic cells. Int Immunol 2005;17:661–9.
- 7 112. Yoshino TP, Wu X-J, Liu H, Gonzalez LA, Deelder AM, Hokke CH. Glycotope Sharing between
8 Snail Hemolymph and Larval Schistosomes: Larval Transformation Products Alter Shared
9 Glycan Patterns of Plasma Proteins. PLOS Negl Trop Dis 2012;6:e1569.
- 10 113. Kwame Nyame A, Leppänen AM, Bogitsh BJ, Cummings RD. Antibody Responses to the
11 Fucosylated LacdiNAc Glycan Antigen in Schistosoma mansoni-Infected Mice and Expression
12 of the Glycan among Schistosomes. Exp Parasitol 2000;96:202–12.
- 13 114. van Liempt E, Bank CMC, Mehta P, García-Vallejo JJ, Kwar ZS, Geyer R, et al. Specificity of
14 DC-SIGN for mannose- and fucose-containing glycans. FEBS Lett 2006;580:6123–31.
- 15 115. van Stijn CMW, Meyer S, van den Broek M, Bruijns SCM, van Kooyk Y, Geyer R, et al.
16 Schistosoma mansoni worm glycolipids induce an inflammatory phenotype in human
17 dendritic cells by cooperation of TLR4 and DC-SIGN. Mol Immunol 2010;47:1544–52.
- 18 116. Boer AR de, Hokke CH, Deelder AM, Wuhler M. Serum antibody screening by surface
19 plasmon resonance using a natural glycan microarray. Glycoconj J 2008;25:75–84.
- 20 117. Frank S, Die I van, Geyer R. Structural characterization of Schistosoma mansoni adult worm
21 glycosphingolipids reveals pronounced differences with those of cercariae. Glycobiology
22 2012;22:676–95.
- 23 118. Naus CWA, Remoortere A van, Ouma JH, Kimani G, Dunne DW, Kamerling JP, et al. Specific
24 Antibody Responses to Three Schistosome-Related Carbohydrate Structures in Recently
25 Exposed Immigrants and Established Residents in an Area of Schistosoma mansoni
26 Endemicity. Infect Immun 2003;71:5676–81.
- 27 119. van Remoortere A, Vermeer HJ, van Roon AM, Langermans JA, Thomas AW, Wilson RA, et al.
28 Dominant antibody responses to Fuca1-3GalNAc and Fuca1-2Fuca1-3GlcNAc containing
29 carbohydrate epitopes in Pan troglodytes vaccinated and infected with Schistosoma
30 mansoni. Exp Parasitol 2003;105:219–25.
- 31 120. Kleij DV der, Remoortere AV, Schuitemaker JHN, Kapsenberg ML, Deelder AM, Tielens AGM,
32 et al. Triggering of Innate Immune Responses by Schistosome Egg Glycolipids and Their
33 Carbohydrate Epitope GalNAc β 1-4(Fuca1-2Fuca1-3)GlcNAc. J Infect Dis 2002;185:531–9.
- 34 121. Martinez-Pomares L. The mannose receptor. J Leukoc Biol 2012;92:1177–86.
- 35 122. Kansas GS. Selectins and their ligands: current concepts and controversies. Blood
36 1996;88:3259–87.
- 37 123. Stacey M, Chang G-W, Davies JQ, Kwakkenbos MJ, Sanderson RD, Hamann J, et al. The
38 epidermal growth factor-like domains of the human EMR2 receptor mediate cell
39 attachment through chondroitin sulfate glycosaminoglycans. Blood 2003;102:2916–24.
- 40 124. Takeda K, Kaisho T, Akira S. Toll-Like Receptors. Annu Rev Immunol 2003;21:335–76.
- 41 125. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-Inflammatory Activity of Immunoglobulin G
42 Resulting from Fc Sialylation. Science 2006;313:670–3.

Figure Legends

Figure 1 Groups of individuals with different sensitization patterns after contact with glycosylated allergens. A lot of people do not develop IgE while other individuals form significant allergen-specific IgE. This IgE is directed against glycans (group A) and/or peptides (group B). While allergy diagnoses for individuals of group B are more and more covered by molecular allergy diagnostics for several important allergen sources, the interpretation of anti-glycan IgE in patient serum is not clear for the allergologist. After the discovery of the allergen α -Gal, patients with IgE against α -Gal (group A1) can be diagnosed correctly, whereas in the past, the delayed-type anaphylaxis to meat and innards was missed by allergy *in vitro* diagnostic tests. However, the allergy diagnosis for patients with IgE to classical CCD of a different specificity (group A2) remains challenging.

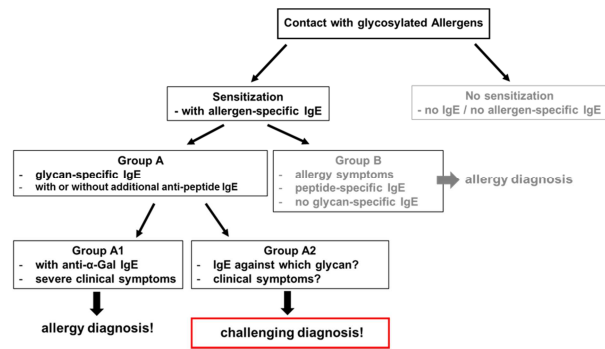
Figure 2 Structure of N-glycans on allergens. A, chemical structure (left) and symbol simplification according to Consortium for Functional Glycomics (CFG) conventions are shown for the N-glycan core glycan. Note that the substitutions of the N-glycan core on allergens are either non-human carbohydrates (xylose) or non-human carbohydrate linkages and positions (α -1,3 fucosylation on the first GlcNAc of the chitobiose core). B, IgE against "classical" CCD are known to cause no or minor allergy symptoms. C, IgE against α -Gal can cause severe allergy symptoms. Non-human carbohydrate substitutions of the N-glycan core are indicated by red brackets. The monosaccharide residues in brackets are present or not in different species. PLA2, phospholipase A2.

Figure 3 Selection of non-human IgE-binding glycan structures from insects, plants, parasites and non-human mammals. Glycans from different species show a high degree of similarity which results in the often observed human anti-CCD-IgE cross-reactivity. Small differences in the carbohydrate structure have pronounced immunologic effects, either foreign monosaccharides like xylose and NGNA or non-human carbohydrate linkages as in the case of α -Gal.

Figure 4 Immunological scenarios for clinical significance or non-significance of IgE. A) Model for the development of high- vs. low-affinity IgE via differential B cell class switch.⁶⁰ B) Activation of Fc ϵ R+ cells via receptor cross-linking is dependent on multivalent antigens (allergens). C) The therapeutic monoclonal antibody cetuximab is particularly effective in IgE binding and Fc ϵ R cross-linking.²³ D) IgE binding to allergens may be blocked by allergen-specific IgG.^{55,56} Consequences of IgG binding to allergens depend on the IgG subclass.

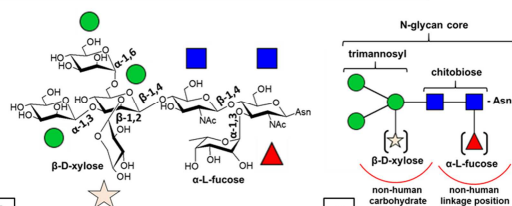
Figure 5 Association of structure and function of foreign and human-like glycans from allergens and parasites. Top: Representative structures of the predominant N-glycans from *S. mansoni* egg antigens (SEA). While IPSE/ α -1 and omega-1 are directing the immune system towards Th2, the N-glycan core of kappa-5 is a target of glycan-specific IgE. No IgE is formed against IPSE/ α -1 or omega-1.^{97,98,100,101} Bottom: Glycans mirroring or mimicking human glycan structures and have an impact on the human immune system which is indicated in the table. s, schistosome; h, human; p, plant; i, insect. References: Le^x,^{49,81,102–104} LDN,^{49,105–112} LDNF,^{49,81,108,111,113–115} FLDN,^{49,116–119} LDNdF.^{117–120}

Figure 6 Immunological effects of selected carbohydrate receptors on human immune cells. Known carbohydrate ligands and the resulting immunological effects are indicated. Top scheme, simplified examples of cellular cross-talk upon receptor interaction with glycans. Attachment and uptake of glycoproteins with specific N-glycans is mediated, for example, by the mannose receptor (MR) or the DC-SIGN receptor. Cross-linking of IgE, e.g. by allergens containing α -Gal, and TLR stimulation by glycan-containing LPS induces pro-inflammatory cellular responses. MR, mannose receptor,¹²¹ DC-SIGN, dendritic-cell-specific intercellular-adhesion-molecule-3-grabbing non-integrin,¹¹⁴ SIGLEC,⁷⁵ selectin,¹²² MGL1¹¹¹, EMR2,¹²³ TLR,^{64,124} Fc γ R.^{74,125}



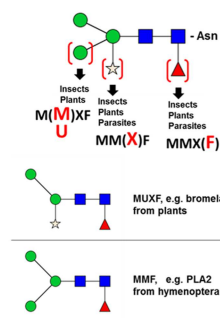
A

Definition and structure of N-glycan core substitutions of allergens



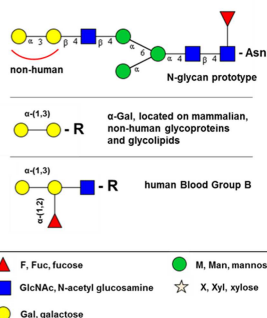
B

"Classical" CCD – minor clinical significance (?)



C

α -Gal – severe allergic reaction



B

"Classical" CCD – minor clinical significance (?)



C

α -Gal – severe allergic reaction



B

"Classical" CCD – minor clinical significance (?)



C

α -Gal – severe allergic reaction



B

"Classical" CCD – minor clinical significance (?)



C

α -Gal – severe allergic reaction



B

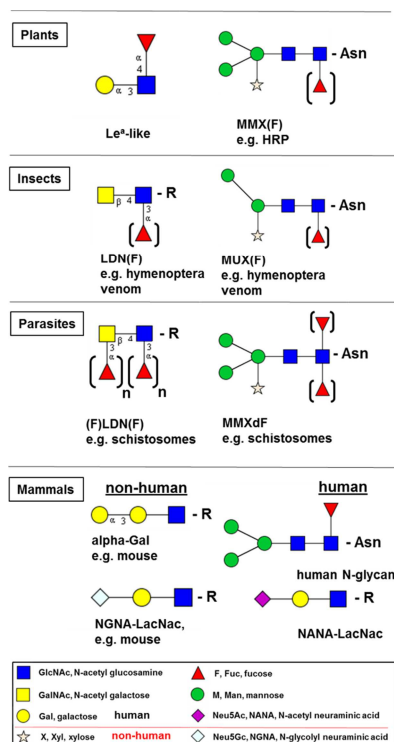
"Classical" CCD – minor clinical significance (?)



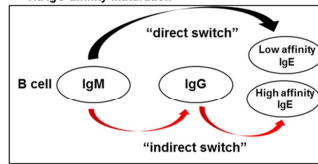
C

α -Gal – severe allergic reaction

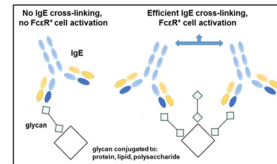




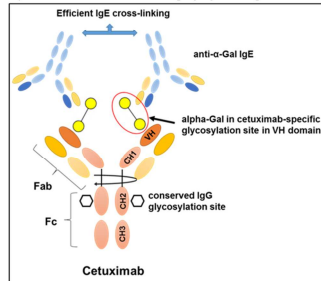
A) High-affinity IgE results from sequential class switch via IgG affinity maturation



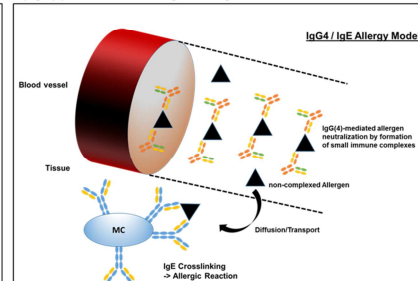
B) Monovalent allergens bind IgE but do not activate FcεR* cells

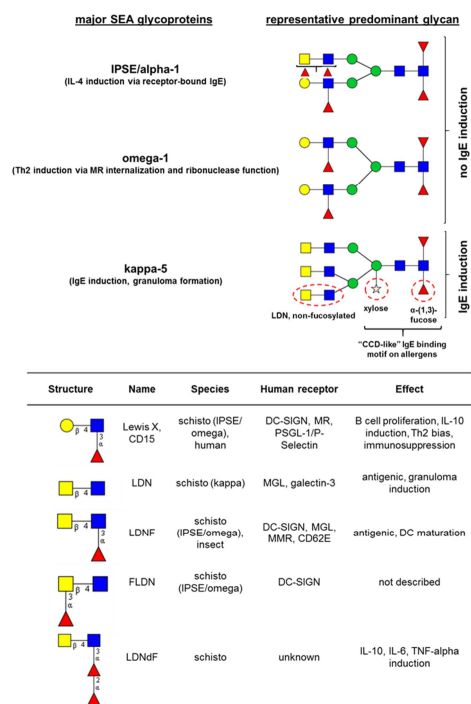


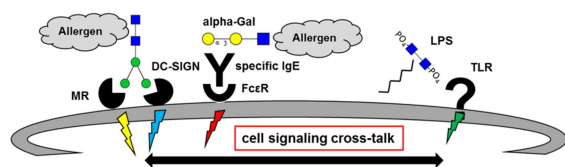
C) Efficient α-Gal cross-linking by specific IgE



D) IgG(4) interference with IgE binding







Human receptor	Receptor class	Carbohydrate ligand (Allergen example)	Cellular expression	Effect
MR/ CD206	C-type lectin	Man, GlcNAc: Fuc (from various allergens, schisto protein omega-1)	mac, mono, epidermal DC	endocytosis
DC-SIGN, CD209	C-type lectin	High mannose (plants, recombinant proteins from <i>P. pastoris</i>)	DC sub	endocytosis
SIGLEC-2, CD22	C-type lectin	sialylated LacNAc (CD75, mammalian incl. humans)	B cell	adhesion, signaling
SIGLEC-7, CD328	C-type lectin	sialylated glycans (mammalian incl. humans)	NK, T sub, mono, gran	inhibits T- and NK-cells
Selectin, CD62E	Selectin	Lewis x (e.g. humans, but also schistosomes)	act endothelial cells	"leukocyte rolling", attachment
MGL1, CD301	Galectin	Gal/GalNAc	mac, DC	cell adhesion, mac migration, cellular recognition
EMR2, CD312	GPCR	Glycosaminoglycans	mac, act, mono, DC, liver, lung	inflammatory response
TLR 2	Toll-like	lipoprotein, glycans	mono, gran, mac, DC, keratinoc., epith.	bacteria and mycoplasma recognition, signaling
TLR 4	Toll-like	LPS (gram-negative bacteria)	mono, mac, endoth., epith., gran, DC	gram-negative bacteria
TLR 6	Toll-like	lipoprotein, glycans	mono, mac, gran, DC, epith.	bacteria and mycoplasma recognition, signaling
FcγRIIa	activating Fc receptor	Gal, GlcNAc	B cells, mono/mac, gran, DC	pro-inflammatory
FcγRIIb	inhibiting Fc receptor	Sialic acid	B cells, mono/mac, gran, DC	anti-inflammatory