Glycotopes as players in the allergic immune response

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Editorial

Asparagine (N)-linked glycosylation is a common modification of secretory and membrane proteins in eukaryotes. However, plants, insects and helminths produce N-glycosylated proteins that carry modifications not found on mammalian glycoproteins. α1,3-linked core fucose residues are present on plant and insect glycans and, in addition, β1,2-linked xylose is found on plant- and helminth-derived glycans**.** Up to 35% of sera of allergic patients contain IgE antibodies specific for glycan epitopes, also termed glycotopes.[1] Initially, these oligosaccharide epitopes were designated as cross-reactive carbohydrate determinants (CCDs) based on the broad *in vitro*cross-reactivity of glycoproteins of plant foods, pollen and hymenoptera venoms.[2] Today, these classical CCDs are listed as group A among 5 groups of oligosaccharides in the official allergen nomenclature website at http://allergen.org/.[3]Although CCDs are probably the most frequent IgE epitopes, their clinical significance is low. This is likely not based on the low affinity of IgE to CCDs, but rather on the affinity of anti-CCD IgGs which function as blocking antibodies.[4] The effect of anti-CCD IgEs are eliminated and false-positive *in vitro* test results are significantly reduced by inhibition of sera with a semisynthetic CCD blocker.[1]

The low clinical relevance of CCDs is in contrast to the effects caused by another glycan, the non-reducing terminal disaccharide galactose-α-1,3-galactose (α-Gal). Anti-α-Gal IgE causes delayed allergic reactions to red meat and immediate anaphylactic reactions to the humanized mouse anti-cancer antibody cetuximab.[5]This reaction is based on the presence of α-Gal on glycoproteins and glycolipids of all mammals, except humans, Old World monkeys and apes. α-Gal belongs to group B of the oligosaccharides included in the official allergen nomenclature website.[3] α-Gal containing glycolipids seem to cause the delay of allergic symptoms after consumption of mammalian meat, since digestion and absorption of lipids is much slower than that of proteins.[6]

In their study in this issue, Plum and colleagues aimed to investigate the interaction of antibodies and CCDs by structural and functional analyses. As CCD-specific IgE producing cells are rare and the establishment of IgE-derived immune repertoires is highly demanding, the authors immunized rabbits with horseradish peroxidase (HRP), a model protein carrying CCDs. They amplified IgG variable regions from the heavy and light chain repertoire from the rabbit's spleen and bone marrow cDNAs and generated a scFv phage display library. Biopanning with CCD-carrying glycoproteins allowed isolating CCD-specific scFv antibody fragments. The use of HRP, a CCD with 3 terminal mannose residues, or HSA carrying MUXF, a CCD with 2 terminal mannose residues, for biopanning resulted in isolation of two different scFv clones (H1 selected with HRP, M5 selected with HSA-MUXF). Both clones were converted into chimeric human/rabbit IgEs and IgGs by fusion of the scFv fragments with human IgE and IgG Fc domains. Previous studies showed that rabbits produce IgG antibodies that bind plant N-glycans with high affinity.[7] However, examples from allergenic proteins, for instance the major grass pollen allergen Phl p 1, indicated that mouse and human IgEs may recognize different epitopes.[8] Therefore, caution needs to be taken when animal-derived antibodies are used as models for interactions between human IgEs and allergens.

The characterization of the chimeric antibodies by ELISA revealed specific binding of the IgE and IgG antibody constructs to the different CCDs and interactions with soluble Fcε and Fcγ receptors. The specificity and sensitivity of the recombinant (r) IgEs were analyzed by ImmunoCAP using CCD-harboring molecules (HRP, HSA-MUXF, and the plant allergen nPhl p 4), extracts from insect venoms (bee, yellow jacket, and*Polistes*), and pollen (rape), as well as extracts from helminths (*Ascaris* , *Echinococcus spp.*) and a nematode (*Anisakis*). The recombinant wasp allergens rVes v 1 and rVes v 5 were included as allergens lacking CCDs. Comparable to human IgEs directed against CCDs, the rIgE antibodies only recognized the CCD-carrying glycoproteins HRP, HSA-MUXF and nPhl p 4 and the CCD-containing extracts from bee and yellow jacket venom and rape pollen. *Polistes* venom, which is devoid of CCDs, was not recognized by the rIgEs.

Surface plasmon resonance analysis revealed high binding affinities between CCDs and rIgEs with K_D values between $8x10^{-7}$ and $8x10^{-9}$, almost in the range of human polyclonal anti-CCD IgE responses with $K_D$values of $1x10^{-10}$.[4] The authors were aware that due to the presence of several CCDs on one protein, such affinity measurements had their limitations. For more accurate measurements it would be important to use glycoproteins with precisely defined carbohydrate moieties.

Basophil activation tests are regarded as reliable diagnostic tools for predicting the clinical relevance of allergens. However, in case of CCDs, the use of these tests as evidence for clinical relevance is questioned, since, for instance, CCD-glycosylated human lactoferrin expressed in rice activated basophils *in vitro* but did not induce symptoms in oral challenges.[9] Plum and colleagues loaded rat basophilic leukemia SX38 cells expressing the human FcεRI receptor with the rIgEs and showed that HRP and the MUXF conjugate could trigger a CCD-dependent ß-hexosaminidase release. Furthermore, binding of rIgE/antigen complexes to CD23 were blocked by CCD-specific IgG from insect venom-allergic individuals. This clearly indicates that human anti-CCD IgEs may *in vivo* play a role in triggering allergic responses.

This study also obtained a crystal structure of a complex between an M5 IgG Fab and the epitope surrogate disaccharide FucαGlcNAcα1-OMe. The disaccharide is recognized entirely by the VH CDRs, specifically, all three hydroxyl groups on the fucose are recognized through hydrogen bonds with CDR1 or CDR2. The authors conclude that the specificity and affinity of the antibody binding is mediated by the α1,3-fucose

and that the fucose residue and the adjacent N-acetlyglucosamine residues represent the crucial part of the glycotope. The future use of such structural biology approaches for analysis of the interactions between antibodies and α-Gal, could contribute to a better understanding in the similarities and differences in the immune response to the two glycotopes.

In summary, the data presented in this study (Figure 1) contribute another important piece of knowledge to the role of anti-CCD IgEs in the Th2 response to glycosylated allergens. The data also point towards glycotopes as players in the allergic immune response.

Word count: 999/1000

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Conflict of interest

The authors declare no conflict of interest.

Figure legend

Figure 1 . **Functional and structural characterization of recombinant IgE antibodies.** Chimeric human/rabbit IgE antibodies were generated by fusion of rabbit anti-HRP scFvs and human IgE constant domains. The recombinant IgEs (rIgE) recognized CCD-containing extracts from bee and yellow jacket venom and rape pollen (1), the CCD-carrying glycoproteins HRP, HSA-MUXF and nPhl p 4 (2), but not the recombinant wasp allergens rVes v 1 and rVes v 5 lacking CCDs (3). The rIgEs caused mediator release from effector cells (SX38) (4). Crystal structures of a complex between the Fab fragment and the disaccharide FucαGlcNAcα1-OMe (Fuc-mNAG) were generated (5) which allowed structural characterization of the interaction between the CCDs and antibodies.