

EDITORIAL

How good are carbohydrates as allergens?

Now that much of the groundwork for the use of recombinant polypeptide allergens or synthetic peptides in the diagnosis and treatment of allergic disease has been undertaken, Yamashita et al. [1] raise the conundrum of glycosylation. Their communication demonstrates that the intranasal administration of glycosylated but not deglycosylated *Aspergillus fumigatus* extract could induce a Th2-type response in mice. The eosinophilic infiltration of the nasal epithelium produced by the response was not associated with anti-carbohydrate antibodies indicating an adjuvant activity. The mechanisms discussed ranged from an increase in allergen uptake by mannose or CR3 receptors on antigen-presenting cells, including dendritic cells to the incorporation of the carbohydrate into the T cell epitopes. A similar phenomenon has been described for *Schistosoma mansoni* egg antigen and recent work by the same authors has shown that human serum albumin conjugated with the carbohydrate of *Schistosoma mansoni* had an increased ability to induce Th2 responses [2]. Although the *Aspergillus fumigatus* extract did not induce IgE anti-carbohydrate antibodies, the ability of plant and invertebrate glycoprotein allergens to induce a high frequency and titre of anti-carbohydrate IgE antibodies has been recognized for two decades [3].

Anti-carbohydrate antibodies to honey bee phospholipase A₂ are found in the sera of most people allergic to bee venom [4] and can sometimes account for 100% of the IgE binding to that allergen. Studies with the sea squirt allergen have shown that the anti-carbohydrate antibodies can be responsible for allergic disease [5] but whether or not this is an exception is a fundamental issue to be discussed below. IgE binding to pollen carbohydrate specificities has been directly demonstrated for Cup a 1 of *Cupressus arizonica* [6], birch extract [7], Lol p 1 [8] and Lol p 11 [9] from rye grass and Ole e 1 from olive [10]. IgE binding to the carbohydrate of timothy [11] and ash tree pollen [12] has been inferred by an association with cross-reactive anti-carbohydrate antibodies. The olive and *Cupressus arizonica* allergens in particular have been reported to be highly glycosylated and to induce a high proportion of anti-carbohydrate antibodies. Recently, a role for carbohydrate antibodies in food allergen has been mooted. IgE to carbohydrate has been found in about 50% of patients allergic to celery [13,14], carrot [15] or kiwi fruit [7] and IgE to cross-reactive glycans have been found in the serum of some patients allergic to zucchini [16], persimmon [17] and tomato [18]. There is also evidence for IgE carbohydrate specificities in mould allergy [19]. Few studies have examined anti-carbohydrate reactivity to common invertebrate allergens, but the potential exists as shown by the

cross-reactivity of IgE from pollinosis patients with caddis fly carbohydrates [20]. The IgE from patients allergic to the caddis fly however, do not bind carbohydrates. Anti-carbohydrate specificities have not been demonstrated for mite allergy although the group 7 and 15 allergens are highly glycosylated [21, 22]. The IgE binding activity of many allergen extracts has been reported to be sensitive to periodate oxidation but the assays do not discriminate between IgE binding to the carbohydrate and the dependence of the polypeptide structure on glycosylation.

Antibody responses to pure polysaccharide antigens are typically T-independent responses with little affinity maturation of the antibodies, but glycoproteins in contrast induce typical T-dependent responses to carbohydrate determinants [23]. IgE anti-carbohydrates are produced against *Schistosoma mansoni* [24] and anti-carbohydrate antibodies to allergens are not limited to the IgE isotype. IgG is produced after immunotherapy with grass pollen extract [25] and IgG anti-carbohydrate antibodies to mucin may also play a role in pigeon fancier's disease [26]. Although the carbohydrate specificities can just be part of the normal spectrum of epitopes they may have special importance because the structures are shared by phylogenetically diverse species and hence anti-carbohydrate antibodies can cross-react between allergens of diverse organisms including plants and invertebrates. This has implications for diagnosis, cross-reactive allergic disease and for epitope spreading.

Analyses of carbohydrate determinants typically begin with the selective oxidation of carbohydrates with periodate. This is a simple procedure for which a number of convenient solid-phase methods have been devised. The assay can be conducted on ELISA plates or on membranes in conjunction with Western blotting. It is however, a time-dependent process and can oxidize amino acids especially tyrosine [27]. Enzymatic methods of deglycosylation provide a range of specific cleavages to determine structure but the reactions need to be conducted in the presence of detergents to increase the reaction efficiency, which could destroy peptide epitopes. Experiments to directly demonstrate antibody binding to carbohydrate specificities employ proteases or heat treatment to destroy the polypeptides or inhibition tests with unrelated glycoproteins known to contain particular carbohydrate structures. More sophisticated experiments use inhibition with synthesized carbohydrates. These analyses have shown that most of the allergens examined have shared antigenic reactivity called the cross-reactive carbohydrate determinants (CCD) [28].

The CCD reactivity has been attributed to the α 1–3 fucose and β 1–2 xylose residues found on the mannose backbone of the glycans [29]. These structures are found on a number of unrelated glycoproteins which are used to test for the determinants. Thus bromelain, plant polyamine oxidase, ascorbate oxidase and horseradish peroxidase are used to demonstrate CCD as

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well as bee phospholipase A. They react with the IgE of subjects with a wide range of pollen and venom allergies and inhibit the binding of IgE to the allergens. The structure of the glycans has been determined for Ole e 1 [30], Lol p 11 [30], Ara h 1 [30], Cup a 1 [31], Cry j1 [32], as well as a tomato allergen [33] and honey bee phospholipase A₂ [34]. The structures are consistent with the known serological cross-reactivities. A comparison of Ara h 1, Lol p 11 and Ole e 1 [30] showed that while IgE binding was directed to α 1–3 fucose and β 1–2 xylose, the reactivity was modified by the addition of other carbohydrates to the backbone. In particular, xylose-specific antibodies which reacted with Lol p 1 and bromelain did not react with a closely related structure with an additional α mannose [1, 3]. No direct correlation was found for IgE binding of individual patients' sera to different allergens and their reactivity to a panel of glycoproteins. The pattern of carbohydrate specificities of the antibodies is therefore complex. Iacovacci et al. [35] produced a monoclonal antibody against the IgE-binding carbohydrate determinant of Cup a 1. It reacted with the pollen from a broad range of plants although it was strongest for related species and quite weak for many others. A complication with studying carbohydrate determinants is that glycosylation has a high degree of microheterogeneity with related structures at different glycosylation sites [30, 34]. Thus, while the main CCD determinants can be approximately defined, the structure will vary.

The evidence to date indicates that IgE antibodies to CCD are not clinically relevant [28, 29]. van der Veen found sera from pollen-allergic patients, with IgE to CCD, bound peanut allergen in RAST assays but the patients did not have positive skin tests or allergic symptoms on exposure to peanut [36]. A low level of peanut-induced histamine release from basophils in *in vitro* assays was however, found. In contrast peanut-allergic subjects had positive skin tests and no IgE anti-CCD. Clearly the CCD did not induce disease. About 50% of patients with allergy to celery and carrot and other food allergens have IgE to CCD [13–18]. A role for these determinants in the allergic reactions has not been excluded but given that the antibodies are only found in half the patients they cannot be the only determinants.

The lack of clinical reactivity attributable to CCD is at least partially due to the fact that they are N-linked glycans. Most proteins only have one N-linked glycan so they would not be able to cross-link the IgE receptors on mast cells and this would be compounded by heterogeneity in the linkage to the mannose core. Of the seven denominated peanut allergens only Ara a 4 has two N-glycosylation sites although Ara h 1 exists as a stable trimer [37] which could also cross-link IgE. The anti-CCD IgE could however, bind other glycoproteins in the extracts and indeed the reactivity to ash [12] and carrot [15] extracts have been shown by Western blotting to be directed at determinants other than the known polypeptide allergens.

The study of Mari found that 41% of the sera from 428 pollen-allergic subjects had IgE to CCD but none of the subjects had skin test reactivity to bromelain which was used as marker glycoprotein [38]. Given that each molecule of bromelain only has one glycan chain this is not unexpected.

In a more general analysis of the requirement for mast cell and basophil activation, it has been shown that the spatial arrangement of the epitopes have a profound effect on the ability to induce degranulation and that multimeric allergens

are more effective than monomers [39]. Studies with multivalent CCD produced by conjugating the glycan from olive [10] or celery allergen [13] to a carrier protein have shown that CCD can induce histamine release. The anti-CCD antibodies are therefore present in sufficient concentration for biological activity so clinical reactivity could be elicited with molecules containing multiple glycans. Ole e 1 and phospholipase A₂ [34] which are associated with high levels of anti-CCD only have one N-glycosylation site [34,40] and thus would not cross-link. Presumably however, the carbohydrate determinants would participate in allergic reactions in concert with peptide epitopes which are also usually univalent. Anti-CCD specificities have been reported to play a significant part in the IgE reactivity of the family of cedar family allergens [6]. Cup a 1 has three N-glycosylation sites [41] and related cross-reactive allergens have two. There is therefore the potential for the determinants to be important within the context of cedar-related allergy. In contrast to the unproven cross-reactivity of carbohydrates, an abundance of allergens with cross-reactive peptide epitopes has been demonstrated [29]. For plants, allergens such as the Bet v 1 homologues, profilins, lipid transfer proteins and thaumatin cross-react across a broad range of species. For invertebrates there are tropomyosin and arginine kinase molecules [42].

As well as mediating the cross-linking of mast cell and basophil receptors, IgE has a function in antigen presentation, which can be via CD23 [43] or via high affinity receptors on dendritic cells and monocytes [44]. The anti-carbohydrate antibodies could therefore act as a focus for epitope spreading where unrelated allergens can be more efficiently presented to T cells. It could also enhance antigen presentation to effector T cells in inflammatory reactions. The high percentage of periodate-sensitive IgE binding activity in the allergens of *Pitysporum ovale* [45], a fungal allergen possibly involved in atopic dermatitis [46], is of interest given the proposed role of the capture of allergen on IgE receptors of Langerhans cells in this disease [44].

The information to date suggests that carbohydrate determinants may, in most situations, reduce the specificity and the diagnostic value of IgE binding tests, and the Th2 promoting activity described by Yamamoto et al. [1] could be counter productive for immunotherapy. Recombinant protein produced in *Escherichia coli* will not be glycosylated and the glycosylation in yeast such as *Pichia pastoris* will not be natural or indeed may be removed during manufacture. The elimination of carbohydrate determinants may therefore be beneficial. However in situations such as allergy to *Cupressa arizonica* pollen and some food allergy the carbohydrate may be important and useful for diagnosis. Similarly, if blocking antibodies have a role in immunotherapy, then the relevance of carbohydrate epitopes cannot be excluded. A recent development has shown the utility of chip-based diagnostic tests presenting an array of recombinant allergens [47]. The pattern of allergen recognition not only gives a clearer picture of the sensitizing allergens but also shows the cross-reacting allergens. This type of test, which would be easier to perform than multiple IgE binding assays, could include recombinant allergens and either allergen-specific or marker glycans to measure cross-reactivities. The clinical importance of the carbohydrate however, remains to be demonstrated.

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