

EDITORIAL

Clinical & Experimental Allergy

Basophil activation test is better but not good enough for the diagnosis of hymenoptera venom allergy: the problem of cross-reactive carbohydrate determinants

This editorial discusses the findings of the paper in this issue by Mertens et al. [3] pp. 1333–1345.

F. Altmann

Department of Chemistry, University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria

Measurement of allergen-specific IgE has for long been the major *in vitro* method for diagnosing allergy and the respective allergen. Certain shortcomings regarding sensitivity (= allergic reactions in the absence of detectable amounts of specific serum IgE) and specificity (= presence of specific IgE that causes no clinical symptoms) have propelled the development of alternative tests. Much hope is laid on near-to-*in vivo* tests such as the basophil activation test (BAT) [1, 2]. Mertens et al. [3] are now revealing that even BAT delivers ambiguous results for patients with hymenoptera venom allergy, i.e. it gives false-positive results with the 'wrong' venom. The reason was clearly identified as sugar linked to some of the allergen molecules. The following treatise will try to explain why this finding is not only annoying but also surprising.

The perception of carbohydrates in allergy began in 1981, when Aalberse reported on IgE directed against a ubiquitously occurring epitope, which he baptized cross-reactive carbohydrate determinant (CCD) [4]. 'Cross-reactive' for immunologists is an attribute in close proximity to 'unspecific' and 'irrelevant'. Thus, the idea of carbohydrates as important players in allergy was for long dismissed by most allergists – at first purely on the basis of ignorance. Around 1990, it became evident that IgE against CCDs was in fact specific for a related group of oligosaccharide structures, all containing a fucose residue in a certain position (Fig. 1) [5, 6]. Such glycans occur widely, in fact almost ubiquitously, on glycoproteins from

plants and insects [7, 8]. At about the same time, it emerged that about $20 \pm 10\%$ of allergic patients exhibit CCD-specific IgE (CCD-sIgE) against these CCDs. Anamnesis of such patients paired with common sense soon pointed at a – fortunately – moderate or lacking clinical potency of CCD-IgE. The observation that peanut allergic patients with IgE against CCD but not against peanut proteins did not react in the skin prick test (SPT) prompted the postulation of 'Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins' [9]. Several other studies, first and foremost that of Mari and colleagues with over thousand patients [10, 11], supported this now widely accepted view, even though a smaller number of his patients exhibited weak skin reactions to one of the test glycoproteins used (i.e. to horse-radish peroxidase). Let us highlight some important aspects of the 'harmless carbohydrate' theory.

(a) CCDs occur in all green plants, in all insects and their venoms, in many other animals but not in vertebrates. This makes them immunogenic.

(b) Carbohydrate-specific IgE elicited by a sensitizing glycoprotein containing CCDs binds to similar glycans on essentially all other glycoproteins bearing the same type of glycans. Thus, such CCD-sIgE containing sera are highly cross-reactive.

(c) No case, where CCDs exhibit clinical relevance has so far been described (even though cautious allergologists keep stating that such cases may exist).

(d) Adding (b) and (c) results in the realization that CCD-sIgE causes false-positive IgE in all types of tests that just measure binding of IgE to allergens.

Starting from the arguable assumption that intra-cutaneous skin-tests reflect the clinical significance of an allergen preparation, Mertens et al. [3] undertook to compare skin-testing, sIgE determination and BAT in order to evaluate the diagnostic value of BAT for insect venom-allergic patients. Apart from a non-allergic control group, there was a group with only yellow jacket

Correspondence:

Friedrich Altmann, Department of Chemistry, University of Natural Resources and Applied Life Sciences (BOKU), Muthgasse 18, 1190 Wien, Austria.

E-mail: friedrich.altmann@boku.ac.at

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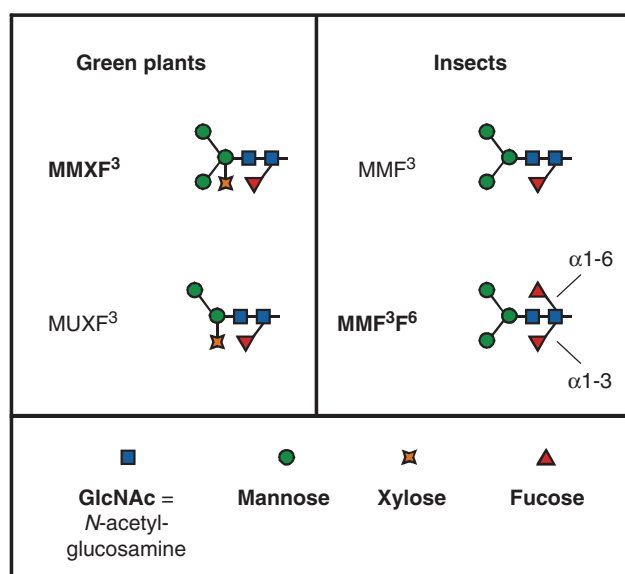


Fig. 1. The cross-reactive carbohydrate determinant family of plants and insects: the MMXF³ structure is an Asn-linked glycan occurring in many plant glycoproteins such as e.g. horseradish peroxidase. MUXF³ is found in bromelain and MMF³ and especially MMF³F⁶ occurs on insect proteins.

venom-positive skin tests, another group with only honeybee venom reactivity and a third group showing skin reaction towards both venoms. Now, if it was perfectly specific, BAT should come up with exactly the same result as the intra-cutaneous skin test. However, roughly one-third of the skin-test mono-reactive patients gave a positive BAT with the wrong venom [3]. Thus, BAT was fooled by CCDs just as traditional sIgE assays, albeit to a lesser extent.

Experiments with insect venoms, where the carbohydrate determinants had been destroyed with periodate, revealed CCDs as the reason for this lack of specificity. Practical consequences of this finding for allergy diagnosis will be discussed at the end of this article.

Before that, it shall be pointed out that the results of Mertens' work contain two most notable details. First, about half of the patients with CCD-sIgE gave a positive BAT, the other half did not. Hence, the serum behaviour in BAT neither complied with intra-cutaneous skin testing nor with sIgE determination. In explicit words: CCD caused a false-positive BAT result for only part of the patients.

Simple explanations for the clinical insignificance of CCD-sIgE such as lacking polyvalency or low binding affinity are either not valid for many allergens (e.g. insect hyaluronidases) or have been experimentally disproved [12]. Another explanation arose from the observation that sera contained more IgG than IgE directed against CCDs, albeit with a somewhat lower affinity [12]. Thus, binding of IgE to glycans occurs under competition with IgG. The

balance may be influenced by repeated contact with large amounts of the determinant as is the case in specific immune therapy or – at no charge – with CCDs in vegetable food. In histamine release tests, this competing IgG is washed away from the passively sensitized basophils [13, 14]. This was a comfortable rationalization for the discrepancy between *in vitro* biological activity by CCD-sIgE and the undeniable lack of *in vivo* activity. The BAT used in the work of Mertens and colleagues, however, uses whole blood and thus the balance between IgE and IgG remains unchanged. This obliterates the competition theory and leaves the 'harmless carbohydrate' enigma on a higher level of incomprehension.

A second point of interest found in Mertens' results is that the periodate-treated venoms gave negative results for a few patients skin-reactive to both venoms. This could point at an active role of CCD-sIgE. These might be the pitiable patients developing allergic symptoms against everything containing polyvalent glycoproteins. Should that really have gone unnoticed? Possibly, these patients were rather cross-reactive on the basis of protein epitopes of a periodate susceptible type. Indication for such unspecific action of periodate was recently found for a mugwort allergen [15]. If I had to bet, I would put my money on 'unspecificity of periodate' rather than on 'true CCD-responsiveness of some patients'. Fortunately, this bet can be decided by scientific experiments.

Why are all these speculations more than mere academic pastime? First, one can expect that an explanation of the clinical irrelevance of CCDs and CCD-sIgE holds an answer for the broader phenomenon of 'IgE without clinical symptoms'. Mertens and colleagues probably show the direction when they state 'It seems that the decision about elicitation of allergic symptoms is not made at the point of cross-linking sIgE-antibodies on the surface of basophils and mast cells but at a different point, e.g. on the level of regulatory T-cells'.

Second, if CCD-sIgE really *never* matters, their misleading influence in allergy diagnosis should be completely eliminated. This can be carried out by using properly-folded recombinant allergens – as long as they are not expressed in insect cells generating CCD determinants [8, 16]. Alternatively, removal of CCD-sIgE or its specific inhibition [17] may confer trustworthy results for insect venoms and many other types of allergy. These measures can be expected to increase the diagnostic specificity of BAT as well as that of sIgE determinations.

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