

EDITORIAL

Clinical & Experimental Allergy

Cross-reactive antigens for natural IgE antibodies: allergens with the potential to transform a dormant to an active allergic response?

This editorial was prompted by the findings of the paper in this issue by S. Blank et al. [1], pp. 976–985.

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Introduction

The study by Blank et al. [1] in this issue is interesting from a number of viewpoints, biological, chemical and medical, covering as it does, major proteins from royal jelly, the intriguing substance with nutritional and cast-determining properties (for bees); the elegant proteomic approaches employed; the recombinant expression of glycosylated proteins by baculovirus infection of moth cells; identification of the royal jelly proteins as venom components; and finally, analysis of the allergenicity of the proteins without interference from attached carbohydrate. The methodology represents impressive state-of-the-art approaches to the study of allergens efficiently applied, but it is the mystery of the so-called CCDs that left one musing. Carbohydrates are not allergens so what is all that carbohydrate-reactive IgE doing there in serum and what is its significance? Not a new question, but highlighted here by the juxtaposition of the apparent insignificance of the carbohydrate antigens and the advanced technology-assisted definition of the allergenic role of the *Apis* proteins. If there are a lot of different carbohydrate antigens that bind with IgE antibodies, are they *all* of no consequence allergenically and clinically?

Antibodies to carbohydrate determinants

Serum IgE antibodies from some allergic patients detect cross-reactions between extracts of various plant-

derived foods, pollens and Hymenoptera venoms. These antibodies, also sometimes induced by Hymenoptera stings [2, 3], are complementary to carbohydrate determinants that have been given the designation cross-reactive carbohydrate determinants (CCDs). The structural basis of the plant and insect cross-reactions are *N*-linked glycans containing oligosaccharides with both *D*-xylose and core α -1,3-*L*-fucose allergenic residues (Fig. 1a) [3–5]. Despite the presence of the CCDs in many potentially allergenic foods, pollens and venoms and the presence of IgE antibodies complementary to these structures in sera of allergic patients, lack of symptoms and negative reactions to CCDs in skin prick tests generally shows that these glycans apparently have no clinical relevance [6, 7].

Although IgE antibodies to CCDs appear to be formed in response to exposure to the various plant materials and venoms, particularly pollens, some of the antibodies are 'natural', that is, antibodies formed without exposure to foreign antigens via infection or passive or active immunization. Certainly, there are natural antibodies to carbohydrate antigens – IgG antibodies in normal subjects with specificities for naturally occurring antigens that the host is constantly exposed to such as normal gastrointestinal and pulmonary bacterial flora. In fact, most of the high titre natural antibodies appear to show anti-carbohydrate specificity and include the anti-blood group A, B and Lewis antibodies (Fig. 1b), antibodies to the cryptic T (Thomsen-Friedenreich)-antigen (Fig. 1c) expressed on the cell surfaces of T-lymphomas and most human carcinomas and antibodies to *N*-glycolylneuraminic acid, a sialic acid thought to be absent in healthy humans [8] (Fig. 1d). One of the natural antibodies found in all individuals tested shows specific recognition of an α -1,3-linked *D*-galactose disaccharide (Fig. 1e) [9], a structure found in the mouse, rabbit, calf and pig, but not in humans

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except for the B blood group antigen, which carries an α -1-2-linked *L*-fucose on the penultimate *D*-galactose (Fig. 1b) that prevents binding to the antibody. A connection between IgE antibodies to the galactose disaccharide and anaphylaxis in humans was recently shown in a most curious way when patients receiving cetuximab, a chimeric mouse-human IgG₁ monoclonal antibody approved for cancer treatment, experienced severe immediate hypersensitivity reactions. The antibodies involved in the reaction were specific for the galactose disaccharide present on the Fab portion of the antibody at asparagine 88 of the heavy chains [10]. Most of the patients who reacted already had the IgE antibodies in their serum before therapy began and the coincidence of intravenous presentation and the presence of the

It has been suggested that the IgE class of antibodies and responses involving it could have evolved as a defence against helminth infection [19], but there has been no epidemiological evidence to support this. In both laboratory animals and humans, helminth infection and high levels of IgE antibodies are not accompanied by allergic symptoms. Nematode-infected rats

actively immune to the parasite are known to reject a challenge infection in the absence of allergic symptoms and, in humans, allergies are uncommon in individuals infected with helminthic parasites. However, when rats and mice are challenged intravenously with parasite antigens, deaths from anaphylaxis commonly occur [20], a scenario similar to the sequence of events and outcome seen with the anaphylactic responses occurring after intravenous injection of the chimeric monoclonal antibody in patients pre-sensitized to the galactose disaccharide. In both cases, the host has gone from a clinically dormant situation where sensitization to an unsuspected antigen exists, only to be exposed catastrophically when the antigen, perhaps in a different guise, is introduced intravenously for reasons quite unrelated to the host's original antigen contacts. As well as human natural antibodies to PC, some antibodies to various parasite antigens are likely to be complementary to PC and of the IgE class. These antibodies might or might not play a beneficial role in patient responses to the parasite but, for the discussion here, what is more pertinent is the possible potential of these antibodies to provoke an immediate type I allergic reaction following an intravenous challenge with an antigen containing an accessible PC determinant or a cross-reactive structure. As already mentioned, C-substances occur widely and contain PC as an immunodominant structure, but their administration intravenously is usually unlikely. Other sources of PC include phosphatidylcholine and sphingomyelin, but in these phospholipid forms in cell membranes, the PC is not exposed for recognition by the immune system unless made accessible by treatment with phospholipase A2.

IgE antibodies to phosphorylcholine and anaphylaxis

There is, however, a likely candidate that might act to convert an apparently clinically dormant allergic state with anti-PC immunoglobulin E into an antibody-mediated lethal anaphylactic response. In the perioperative situation, pre-existing IgE antibodies of unknown origin in the sera of some patients receiving a neuromuscular blocking drug (NMBD) for the first time can mediate a life-threatening anaphylactic reaction. The antibodies, specific for tertiary and quaternary substituted ammonium ions [21], detect these groups on NMBDs such as succinylcholine (Fig. 1g) and atracurium and the drugs, by virtue of at least two of these well-separated ions, are able to flexibly cross-link and activate mast cells to release the mediators of anaphylaxis. The specificity of the antibodies equips them to react readily with PC and it was recently suggested [22] that this quaternary ammonium compound might be the stimulating antigen and natural antigenic complement for the IgE antibodies responsible for the NMBD-induced allergic reactions

during anaesthesia. Figures 1f and g show the identity in structure of the extended quaternary ammonium groups on PC and the identified allergenic terminal groups widely separated at each end of succinylcholine [21, 22].

From the inactive to the active allergic state – what are the mechanisms?

With the foregoing examples in mind, we can return to the CCDs where, at the moment, we do not have enough information on the variety of specificities likely to be involved in IgE responses, although we can already be sure of the occurrence of antibodies to a range of diverse structures from simple sugars such as *D*-galactose, *L*-fucose, *D*-xylose and *D*-mannose in oligosaccharide or glycoconjugate form to neuraminic acids and PC from glycolipids. Viewed from this perspective, the intravenous administration of any new therapeutic recombinant protein or other agents that might have some structural features in common with component entities of glycoconjugates should be critically examined for their potential to elicit an unsuspected serious allergic response. If the above interpretations and speculations are correct, an explanation is still needed for the benign nature of the humoral response to the CCDs with its absence of clinical symptoms and its contrast with the predicted allergic activation following intravenous presentation of the appropriate antigen. Interference with IgE antibody binding by blocking antibodies such as IgG₄ is utilized in desensitization injections (immunotherapy) for the treatment of common allergies and protective antibodies to CCDs have been demonstrated in sera of patients receiving grass pollen immunotherapy and in patients occupationally exposed to bovine and porcine proteins [23]. Evidence that IgG₄ antibodies may block IgE pathways is seen in the delayed development of protective IgE antibody-mediated immunity in schistosoma infection in humans [24, 25]. A further question that needs addressing is why should the route of antigen challenge make such a difference in transforming the apparent absence of allergy to a clear type I hypersensitivity response? This is clearly demonstrated in parasite-infected laboratory animals and in the human examples discussed here, but apart from the well-known fact that anaphylaxis is a key risk of peripheral intravenous therapy and the parenteral route of administration increases the severity and frequency of an anaphylactic reaction, there seems to be no readily apparent explanation. Excluding the possible involvement of blocking antibodies, the absence of allergic clinical symptoms to CCDs and the uncharacteristic delay in anaphylaxis following red meat ingestion may be the consequence of digestion of the antigen and/or processing by the

immune system. This is not a consideration with intravenous administration where the antigen is delivered rapidly undigested and unprocessed ready to interact immediately with IgE antibodies in the serum and on mast cells.

In conclusion, there are indications and some evidence that CCDs as oligosaccharides and glycoconjugates, and PC found in some glycolipids, should be viewed, until proven otherwise, not just as potential allergens for inducing allergic cross-reactivities between foods, pollens and venoms, or as clinically insignificant antigens causing false-positive results in *in vitro* IgE antibody

assays, but as potential allergens that are part of a dormant allergic response 'waiting' as it were for the right stimulus via the right route to activate their involvement in a full-blown immediate hypersensitivity response. Evidence for this conclusion is clearly still incomplete, for example, if blocking antibodies are involved, what mechanisms overcome the block? Future research on mechanisms underlying allergic recognition of CCDs carried out with this perspective in mind may lead to some new insights and reveal the correctness or otherwise of the interpretations and predictions outlined here.

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