

Clinical relevance of carbohydrate allergen epitopes

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This celebration of the 30th anniversary of the discovery of IgE reflects the importance of this minor immunoglobulin isotype. The cell-activating potential of IgE is undoubtedly its most striking property and explains its importance in allergy diagnosis (3, 4, 8). In this paper, I will discuss the exception to this general rule that the interaction between allergen and cell-bound IgE antibody results in the potent activation of cells. My topic focuses on IgE antibodies that seem to lack this characteristic high efficiency and virtually fail to induce cell triggering upon contact with the matching antigen. The prototypes for this category of low-activity allergens are nonmammalian glycoproteins with IgE-binding carbohydrate epitopes. This topic is particularly relevant in relation to false-positive serology: IgE antibodies reactive to foods in the absence of overt food allergy (a long-standing issue in allergic serology (10, 18, 20)).

I will use the term “glycan” for any carbohydrate structure, in the form of either a polysaccharide or the sugar-chain(s) of a glycoprotein. Since IgE antibodies to nonmammalian glycans usually prove to be highly cross-reactive (i.e., these antibodies do not discriminate between similar glycans on very different protein backbones), the term “cross-reactive carbohydrate determinant” (CCD) is used. For two recent reviews on CCDs, see Aalberse & van Ree (1, 2).

Carbohydrate epitopes are in several ways different from “classical” peptide epitopes. Immunologically relevant differences are found both in the structure of the epitope and in the way the immune system interacts with the epitope.

Carbohydrate epitopes and peptide epitopes: structural differences

In contrast to (most) peptide epitopes, carbohydrate epitopes are not directly genetically encoded. Their structure is determined by the concerted activity of enzymes (glycosyl-transferases, trimming glycosidases, and other modifying enzymes such as acetylating and sulfating enzymes). Moreover, glycans are usually branched, substantially increasing the structural complexity. Thus, even if the number of different building blocks in peptides and glycans (amino acids and monosaccharides, respectively) is lower in allergologically relevant glycans, variability is very substantial. Antibodies may sometimes be highly specific for a particular glycan. This is especially the case for glycans that are produced by mammals. For nonmammalian glycans, however, antibodies to glycans are often less discriminating and cross-reactivity between distantly related glycans is very common in this situation.

Glycans have little, if any, secondary structure and are therefore resistant to denaturation. As a consequence, carbohydrate epitopes are very efficiently detected on immunoblots.

Carbohydrate epitopes and peptide epitopes: differences in immunologic recognition of the epitope

For antibodies, there is no essential difference between peptidic and glycosidic epitopes. In contrast, the classical MHC molecules bind only

peptides in the groove; thus, T cells tend to react to peptidic epitopes (with possibly a glycan attached). This explains why immune responses to pure glycans are T-cell independent and why antibodies that are induced by pure glycans have a restricted isotype pattern (in man, mostly IgM and some IgG1 and IgG2). Since the isotype switch to IgE and IgG4 requires the prior activation of T cells, it is often assumed that antiglycan antibodies are not found in these isotypes. This is true only in the case of antigenic stimulation by pure glycans, but not if the antigen is a glycoprotein. This situation is analogous to the classical hapten-carrier immunization, with the glycan-part acting as a hapten and the peptidic part of the glycoprotein as the carrier that will activate T cells. As with hapten-carrier systems, the antibodies often do not require the presence of the carrier to interact with the sugars and will react with these sugars on an unrelated "carrier". In some instances, however, the "carrier" influences the binding affinity.

It is important to stress that T-cell-dependent antibodies to carbohydrate epitopes are not uncommon. The existence of such IgE antibodies is thus not at all unexpected, and the observed cross-reactivity of such IgE antibodies is fully in line with classical immunology.

IgE antibodies to carbohydrate epitopes appear to lack biologic activity

IgE is noticeable because of its exceptional efficiency in a particular type of biologic activity: antibody-dependent cell triggering. However, it seems that not all IgE antibodies are equally effective in this respect. In the next section, I will discuss some of the mechanisms that might be involved in variability of IgE-allergen effectiveness.

Structural variability in IgE

Structural variability has been described for IgE. Marked variability in glycosylation patterns has been noted, both by analysis of reactivity to lectins and by chemical analysis of myeloma proteins (31, 32, 44). Variability in reactivity to staphylococcal protein A probably reflects the VH subgroup that the IgE uses (11, 13, 14, 21). Variants of IgE due to different splice site usage have been described, but it is uncertain to what extent these splice variants are secreted by plasma cells. The relative biologic reactivity of these structural variants of IgE are mostly unclear as yet. Also unclear is the structural basis of differences in biologic reactivity (based on reactivity to IgE-dependent histamine-releasing factors) described as IgE⁺ and IgE⁻ by MacDonald et al. (24–26); it might reflect one of

these structural variations, but data currently available do not rule out the possibility that IgE⁺ is a conventional IgE antibody to an autoallergen (HRF) (30).

Modifying effects of concomitant antibodies

The effects of IgE antibodies may be modified (enhanced or suppressed) by other antibodies. These effects would show up *in vitro* upon comparison of allergen-induced mast-cell/basophil activation in the presence or absence of plasma. Downregulation would be expected from classical blocking (allergen-neutralizing) IgG, but also from FcRIIb-activating IgG antibodies to the allergen, even if these latter antibodies would not directly compete with IgE for epitopes on the allergen (6, 7, 23). It is likely that these effects explain the absence of anaphylactic effects in the large majority of subjects after vaccination with tetanus toxoid (despite the often substantial levels of IgE antibodies to the toxoid). Enhancing activity would be expected from cross-linking IgG anti-allergen antibodies (34) and from IgG (4) anti-IgE (5, 9, 12, 15–17, 19, 22, 27–29, 33, 35–41, 43).

Allergen-dependent variability

Two important determinants of antigen-induced cell triggering are affinity and valency. There is still remarkable uncertainty on both issues in relation to IgE-mediated reactions. Monoclonal mouse/human chimeric antibodies to the mite allergen Der p 2 with dissociation constants in the low nanomolar range were effective; two antibodies were sufficient (34). However, it still has to be established whether antibodies with lower affinities are effective and whether combinations of more than two antibodies are more efficient.

How about IgE to CCD?

From the theoretical point of view

Information on the mechanism or mechanisms that explain why these IgE anti-CCD antibodies are inefficient in inducing symptomatology is urgently needed, not only for the CCD-related problems, but also because such information might give clues to the modulation of IgE-mediated reactivity to other allergens.

From the practical point of view

Clinicians should know about IgE to CCD, because these antibodies tend to cause confusion. They

react to extracts of almost any vegetable food, but are often found in sera from subjects without signs of food allergy. We investigated reactivity of IgE to CCD in patients with a positive peanut RAST without peanut-related symptoms (42). Of 173 patients that were sensitized to grass pollen (SPT and RAST), 50 were found to be positive in the peanut RAST, but negative in the peanut SPT; they had no peanut-related allergic symptoms. Of these 50 cases, 32 were further investigated. IgE to CCD was found in 29, and, in these cases, IgE binding to peanut could be attributed to CCD. In contrast, IgE from patients with peanut allergy did not react to CCD. Peanut-induced histamine release from basophils was markedly lower (but not completely absent) in the CCD-positive subjects (42).

These results support the concept that the accuracy of serologic allergy tests will improve if CCD-related reactivity can be avoided.

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