

Structural aspects of cross-reactivity and its relation to antibody affinity

In the context of IgE/allergen interactions, affinity is largely determined by the stability of the allergen-IgE complex: a low affinity is usually equated with a rapid dissociation of the complex. Regular solid-phase assays are not well suited for affinity estimates because of multivalency effects, “unstirred layer” effects and “invisible” antibodies. Elution of IgE bound to solid-phase coupled allergen might be a good measure of intrinsic affinity, provided that reassociation of antibodies is prevented by a high concentration of soluble allergen. Allergen-mediated IgE-dependent triggering of a mast cell is presumably a two-step process. During the first step, the allergen is bound to a cell-bound IgE antibody and dragged over the cell surface. The second step is the interaction between this cell-bound allergen and another IgE antibody. The hypothesis is that the affinity requirements for the first step are higher than for the second. The implication is that a mast cell can be triggered by a single high-affinity antibody in combination with one or more low-affinity antibodies.

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Cross-reactivity is an immunological issue: it only exists in relation to antibodies (and T cell receptors, but this review will be restricted to antibodies). We may say that two proteins are cross-reactive if there is at least one antibody that binds to both these proteins. Since it is impossible to test the whole universe of all antibodies of all invertebrates, it is impossible to prove that two proteins are NOT cross-reactive. This definition of cross-reactivity is incomplete, because it does not specify the strength of the binding. In some situations even a transient interaction may be of interest, but usually the affinity of the interaction needs to exceed some threshold value before it has any biological effect. This affinity threshold is variable in time and localization: if the cell happens to be in a primed state, the affinity threshold may be lower (or higher).

In the case of allergens, at least three biological effects have to be distinguished: triggering of T cells, triggering of B cells and triggering of mast cells. The issue of cross-reactivity is different in each of these three situations. Since this short review is focused on antibodies rather than on TCRs, only the two antibody-dependent systems (B cells and mast cells) will be discussed (see also 1).

Affinity and avidity

Mathematically, affinity is defined as the quotient of the association rate and the dissociation rate of antigen and antibody. The present discussion is concerned with

similarly sized globular antigens. In this context, differences in affinity can be considered to be largely a reflection of differences in the rate of dissociation, or the half-life of the antigen-antibody complex ($t_{1/2}$, ranging typically from 1 min to 1 day).

The intrinsic affinity of an antigen/antibody combination corresponds to the interaction between a single antigen-binding region of an antibody and a single antigen molecule. Two effects may give rise to a markedly higher stability of an antigen-antibody complex: multivalent attachment and the “unstirred layer”. This higher overall stability is referred to as “avidity”.

The effect of multivalent attachment is well known. If a ligand is fixed at two attachment sites, dissociation at one of the sites is more likely to be followed by reassociation rather than by dissociation of the 2nd site. This cooperative process thus leads to a marked increase in avidity.

The second process is largely similar, but does not depend on multi-site physical interaction. Macroscopically, a complex may behave as being more stable than it is on a micro-scale. In some situations, antigen may dissociate from the antibody (or the other way around), but very soon after this dissociation it may reassociate. This rapid reassociation becomes more likely if either the antigen or the antibody is on a surface (either a cell surface or an immunoadsorbent surface, e.g. in a RAST assay or ELISA plate). This effect is stronger if the density of the

reaction partner on the solid phase is high. If the antigen is physically unattached to the antibody, but is much more likely to reassociate than to diffuse out into the medium, it is said to be in an “unstirred layer”.

The difference between affinity and avidity can be investigated experimentally by comparing the rate of dissociation in the absence or presence of a reagent that prevents reassociation, e.g. by measuring the dissociation of antibody to solid-phase bound antigen in the presence or absence of a large excess of antigen (Kleine Budde et al., manuscript in preparation). The difference between multivalent attachment and effects due to the “unstirred layer” can be investigated by comparing monovalent and multivalent reagents (e.g. Fab fragments of antibodies versus intact antibodies or F(ab)₂ fragments).

How to measure affinity and avidity of IgE-allergen interactions

The literature on the subject of affinity/avidity measurement is extensive, but much of it is not easily applicable to IgE-allergen interactions. One issue is the effect of “invisible” antibodies, i.e. non-IgE antibodies reactive with the allergen. This hampers, for example, the interpretation of procedures in which allergen is incubated with serum, such as direct RAST or RAST inhibition. A procedure to measure avidity in a solid-phase system using direct binding in the presence of “invisible” antibodies has been described elsewhere (2). Such a direct-binding procedure still has the disadvantage that the avidity depends on the type of solid phase and by multivalency (see above). Using a reversed RAST system, with a solid-phase coupled anti-IgE in combination with labeled allergen, interference by the “invisible” antibody can be avoided by washing the solid phase before the allergen is added. But, again, the avidity effects of a solid phase have to be considered.

An attractive alternative is to measure elution of IgE using a high concentration of antigen (see ref. 3 and Kleine Budde et al., manuscript in preparation).

Cross-reactivity and B cells

Similar to mast cells, the allergen has to crosslink two surface-bound antibodies in order to trigger a B cell. However, in contrast to mast cells, the antibodies that are responsible for the triggering of a B cell are monoclonal. For a typical allergen (i.e. a small monomeric protein without repeating epitopes) this obviously poses a problem. There are different solutions to this problem, all basically involving a crosslinking third partner. This third partner can be another cell to which several allergens are attached. Alternatively, it can be a crosslinking antibody with a non-overlapping epitope specificity. If, for example, the allergen has two non-overlapping epitopes, A and B, it may trigger a B cell with antibodies with specificity for epitope A if two

allergen molecules are bound to a bivalent antibody to epitope B. This immune complex can either be soluble, or be bound to a follicular dendritic cell.

This situation may well be relevant in relation to cross-reactivity. A first contact with the primary sensitizer (for example, profilin from grass pollen) induces a wide range of antibodies (different isotypes and different epitope specificities). By way of illustration we assume that among these antibodies there are IgG1 antibodies to a well-conserved site X_{grass}. Upon subsequent contact with profilin from carrot, with a closely similar epitope X_{carrot}, these IgG1 antibodies might form immune complexes with carrot profilin and present this immune complex to a B cell with antibodies on its surface to a carrot-specific (non-cross-reactive) epitope Y_{carrot}. This may trigger the B cell to produce antibodies that are specific for carrot profilin (and are not reactive with grass profilin). This process, cross-reactive priming, might be responsible for broadening of the antibody repertoire.

The above is largely hypothetical. Actual evidence that this process is relevant in clinical allergology is scarce. Experimental evidence should come from cross-inhibition studies. In the above example one would expect to find antibodies to the secondary allergen (carrot profilin) that cannot be inhibited by the primary allergen (grass profilin). In most cases where this has been tested, a primary allergen can be identified (usually pollen profilin in the example) that will completely inhibit all the cross-reactive antibodies.

This may have to do with affinity as well as antigen/antibody ratio. For cross-reactive priming, the secondary allergen (carrot profilin) has to form stable immune complexes that are either large enough to adhere to the follicular dendritic cell or of the type Ag₂Ab (i.e. antigen excess) in order to be able to crosslink the monoclonal antibodies on the B cell surface.

Cross-reactivity and mast cells

The classical picture of allergen-induced mast cell triggering shows the allergen fixed to the surface of the mast cell via two IgE antibodies. The antibodies are implicitly assumed to be equivalent. The actual sequence of events is likely to be different. The allergen is first anchored to the mast cell surface by the first IgE antibody. This IgE-allergen complex then moves over the cell surface until it meets a second IgE antibody with the proper specificity (i.e. reactivity to a non-occupied epitope on the allergen surface). The affinity requirements for these two processes are likely to be different. In particular, it is likely that the first interaction requires a stable attachment, whereas the second interaction only has to last for a short time and thus would occur even with antibodies of low affinity.

Cross-reactivity and affinity

The interaction between an antibody and the primary antigen usually (but not always) has a higher affinity constant than that with the secondary (cross-reacting) antigen. More distantly related antigens will usually (but again, not always) show weaker cross-reactivity. So, the question is, when does cross-reactivity become insignificant and how does this threshold differ in serological assays and in bioassays? In most serological assays cross-reactivity is measured with a relatively high concentration of solid-phase bound antigen (RAST, immunoblot). It should be realized that a 10-fold loss in affinity can be completely compensated for by a 10-fold increase in the concentration of one of the reagents. Therefore, if the allergosorbent (or the immunoblot) has a sufficiently high allergen density, IgE antibodies of relatively low affinity will be detectable. Titration of antibody is far more common than titration of allergen.

In contrast, for bioassays it is customary to titrate the antigen. It is therefore not surprising to find discrepancies between cross-reactivity patterns in both types of assays. One option is to titrate allergen rather than antibody even in serological assays (4). In principle, this can be done conveniently with fluid-phase allergen, but the results may still be influenced markedly by multivalency and “invisible” antibodies (mainly IgG antibody). The alternative procedure, measuring the elution rate of bound IgE antibody in the presence of a large excess of soluble allergen (to prevent reassociation), might be a better alternative.

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