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Improving allergy outcomes

Liquid Allergens improve in-vitro allergy diagnostic value

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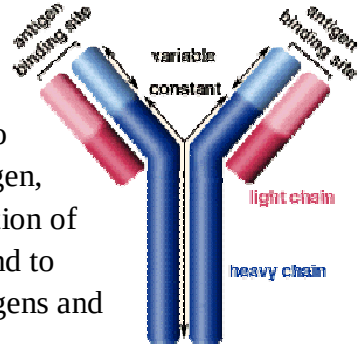
Photo by Jenny Downing

Since the discovery of IgE, serum testing for allergen specific IgE has been used in allergy diagnosis. The RadioAllergoSorbant Test (RAST), first described by Wide et al. in 1967[1], has been enhanced over the years using new technology to improve the diagnostic value of the test. Most diagnostic manufacturers have continued to use solid-phase allergens. At first the solid-phase was a paper disc and more recently a cellulose sponge has been used for coupling to allergens. Allermetrix uses an additional improvement to the RAST assay, liquid allergens instead of covalently binding allergens to a solid-phase. Liquid allergens represent a technological breakthrough that improves assay performance and the diagnostic value of the specific IgE test to a clinician.

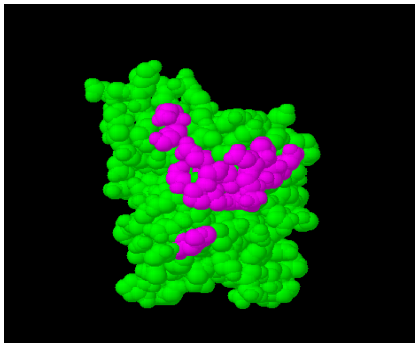
Allergens are proteins that induce IgE response in predisposed individuals. Allergens cause the activation of mast cells when IgE specific to the allergen is bound to the surface. The section of the protein that the IgE antibody binds is called the epitope, or antigenic determinant. The area of the antibody that binds to the epitope is called the antigen binding site, combining site, or paratope. The combining site is always found in the same area of the antibody, and there are 2 identical paratopes on each IgE antibody. In order to activate a Mast cell, at least two bound IgE antibodies must crosslink an allergen, which indicates that allergens must have multiple epitopes. Clearly the condition of the epitopes in the specific IgE assay will affect the ability of serum IgE to bind to them. It is the structure of these epitopes that is well preserved as liquid allergens and not as solid phase allergens.

Figure 1 Antibody paratope or antigen binding site depicted on model of antibody molecule



Epitopes have been evaluated for many years by immunochemists and can be broadly characterized into two groups. Linear (continuous determinants) consist of a chain of amino acids that can be shown to react to the combining site of an antibody. Discontinuous (conformational determinants) have at least 2 parts of the protein amino acid chain or chains that are not contiguous required for antibody binding. Generally linear determinants do not require that native protein 3-dimensional structure remain preserved. For antibody binding to conformational epitopes, 3-dimensional structure must be present in the protein.

Figure 2 Depiction of a conformational determinant on the allergen Bet v 1. Purple residues indicate binding site of anti-Bet v 1 antibody.



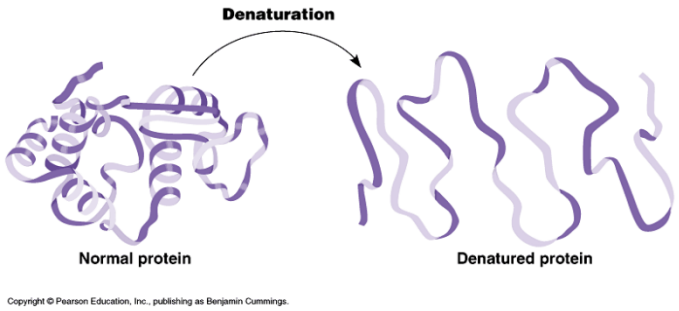
Linear epitopes are commonly evaluated by synthesizing peptides that cover the whole amino acid sequence of a protein with overlapping sequences in each peptide. In this way, each sequence may be present in many peptides. Antibodies from allergic individuals are tested to see if they react with any of the peptides. Peptides that demonstrate reactivity are then further evaluated for inhibition of the native IgE binding to the whole protein. The stronger the antibody inhibition the more likely the peptide represents an epitope of the antigen, or in the case of IgE antibody, allergen.

Conformational epitopes can be demonstrated by the ability of antibody to bind naturally occurring proteins and not denatured or damaged proteins. Sachs et al.[2] cleverly showed that 2 peptides which alone were random coils did not bind antibody from immune serum. However, when mixed together in solution the peptides could be shown to complement each other and form secondary structure, binding antibodies from antiserum. These antibodies had a binding site that required a specific 3-dimensional structure of the antigen to react. It has been estimated that as many as 90% of all antibodies are of the conformational type[3,4]. In one report, immunization in PBS without adjuvant, similar to natural exposure, elicited antibodies to conformational determinants of the immunogen which reacted poorly with denatured immunogen[5]. It has also been shown that conformational determinants are required to initiate food allergy responses, and can break tolerance in patients who have outgrown the disease[6].

In a recent study using mosaic, genetically engineered, allergens[7], allergic patients serum binding to native allergen was reduced an average of 86.5% (range: 65.4 to 96.4% reduction) when tested with the mosaic protein. One mosaic protein was genetically engineered by breaking the DNA of the original protein into 4 different pieces, each approximately ¼ of the original protein and assembled in a DNA construct that was transcribed into a single protein. This mosaic protein had the same amino acid composition, and each peptide sequence remained the same as the native protein. However, the new mosaic protein had the peptides out of order. The mosaic construct would most likely have a different 3-dimensional structure than the native protein, but have many linear portions identical to the protein. The poor binding of serum from 28 allergic donors to the native protein supports the importance of conformational determinants for detecting relevant IgE.

Allergens used in specific IgE assays are processed in various ways. Solid-phase allergens can be passively adsorbed onto a plastic surface such as polystyrene, or covalently attached to a matrix such as cellulose. It has been clearly demonstrated that solid-phase adsorption onto plastic surfaces denatures proteins[8,9]. Chemical allergen linkage to cellulose has also been shown to denature proteins and reduce immunoreactivity[10]. In contrast, biotinylation of proteins has been demonstrated to maintain natural protein conformation and have 20 – 30 times the immunoreactivity of a solid phase allergen[5,10].

Figure 3 Representation of protein denaturation as occurs on solid phases used for allergen testing.



Non-specific binding (NSB) in an immunoassay is the amount of positive signal created by a negative sample. In assay design the lower the NSB (background) the better the ability to detect small amounts of the analyte, which is allergen-specific IgE in these assays. Liquid allergens have very low non-specific binding in contrast to solid phase allergens that have a volume of matrix associated with the covalently bound protein. Matrices such as cellulose have higher background binding, which reduces the analytical sensitivity of the assay and may trap aggregates or immune-complexes that may be present in a patient's serum[9]. Anti-IgE – IgE immune complexes have been detected in patients with asthma, chronic urticaria, atopic dermatitis, various autoimmune diseases and even normal patients[11,12,13]. Such immune-complexes may increase cross-linking due to cooperative effects of solid phase systems[14].

Several reports have demonstrated the advantages of liquid phase allergens over solid phase allergens. In one report[10] Aalberse et al. demonstrated that liquid phase allergens gram for gram, bound 20 – 30 times more than the same allergen on solid phase. When compared to a commercial specific IgE assay, the liquid phase allergens bound more IgE than the corresponding solid phase allergens in the commercial kit. In a another report[15] 2 of 3 patients allergic to milk, who had specific IgE to beta-lactoglobulin, a milk protein, bound more IgE to the liquid phase allergen than to a solid phase form of the same allergen. Liquid allergens preserve the relevant epitopes for detecting IgE in allergic serum.

Allergen specific IgE is found at very low concentrations in serum and the *in vitro* assay sensitivity is extremely important to clinical performance. As a result, low non-specific binding is an important attribute of any assay. Allermetrix specific IgE has an analytical sensitivity of 0.05 kU/L compared to the leading commercially available solid phase assay which has analytical sensitivity of 0.11 kU/L[16].

Another type of undesirable binding may be related to samples. In one study, the market leading commercial solid phase assay was compared to skin testing for 53 different allergens in 131 patients with chronic rhinitis whose total IgE level had been determined. One finding was that patients with a total IgE > 200 kU/L (high total IgE) had on average more in vitro than skin test positive results per patient, 26.5 vs. 21.5 respectively. In the patient group with total IgE levels < 200 kU/L (low total IgE), in vitro had an average of 8.3 and skin testing 15.3 positive results per patient. For 52 of the 53 allergens tested, there were statistically significantly more positive *in vitro* results in patients with high total IgE than low total IgE. Only 14 of the allergens demonstrated the same significant difference between high and low total IgE patients when evaluated by skin testing. The increase in 98% of the allergens binding due to high total IgE was not expected. The authors suggest that the patients may have circulating IgE that is not clinically reactive in the skin. Another explanation may be that the cellulose solid phase system does not perform properly when allergic patients have elevated total IgE levels.

As discussed above, anti-IgE – IgE immune complexes are often present in the sera of patients with several allergic conditions, and it has been demonstrated that the higher the total IgE level, the more likely they form[17]. Since these immune complexes are susceptible to capture by solid

phase matrices, one might deduce that the increased rate of positivity in vitro with cellulose solid phase systems is not due to increased specific IgE, but rather IgE that is not specific for the allergen tested. In this study, the behavior of solid phase cellulose systems is not consistent with skin testing performance and seems more likely due to an inherent system characteristic because 98% of the allergens tested exhibited the behavior. Liquid allergen systems are not susceptible to this problem because there is no matrix to hold up non-specifically bound IgE.

Liquid allergen assays have practical, technical and clinical advantages over solid phase assays. Practically, liquid allergens enable automation applications not available for solid phase systems which significantly reduce operational errors. Technically, they have high analytical sensitivity, low non-specific binding and preserve the natural conformation of the allergens. Clinically, liquid allergens measure relevant antibodies to conformational epitopes. All together liquid allergen immunoassays provide physicians the most reliable information available to support the diagnosis of allergy.

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