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Improving allergy outcomes

Allergen Component Testing

Jay Weiss Ph.D and Gary Kitos, Ph.D. H.C.L.D.

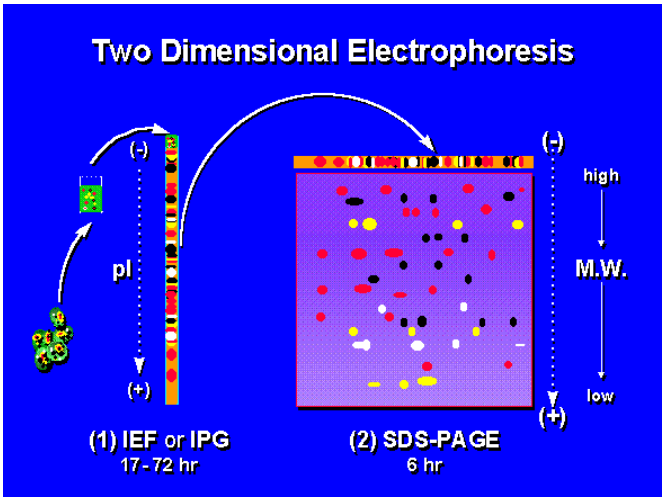
Allergen Component Testing

Allergic disease is an immunologic response to an allergen or allergens that cause immediate type reactions that include sneezing, itching, difficulty breathing and anaphylaxis. In 1967 the antibody responsible for these reactions, immunoglobulin E (IgE) was discovered and promised to revolutionize the diagnosis and treatment of allergy. A modality of diagnostic testing was launched, allergen specific IgE serology, and a treatment, anti-IgE therapy. However,advances in allergen immunotherapy have lagged, because allergens are complex, variable, and difficult to dissect into their components. Beginning in the 1970's, individual allergen proteins began to be recognized and isolated. With the advent of molecular cloning and proteomics, individual allergens have been identified, purified, and engineered as recombinant proteins. Many allergen components have been extracted from the complex mixture of proteins found in allergen extracts. These purified components offer new ways of analyzing and altering the immune response of allergic individuals.

Allergen extracts that are currently used for immunotherapy and diagnostic testing are generally a crude mixture of proteins that are mostly not allergens. Immunotherapy with purified specific proteins would eliminate a patient's exposure to irrelevant allergens during immunotherapy, and target the specific cause of reactions. Additionally molecular engineering of purified allergens has been shown to reduce potential allergenic response while maintaining immunogenic activity.[1] An effective non-allergenic immunotherapy vaccine would be a major breakthrough in curing allergy. For such therapy to become reality, the immune response of allergic patients will need to be better understood.

Assesment of patient sensitivity to specific allergens can be conducted in many ways. Today, the skin prick test and allergen specific IgE are the most common methodologies used by clinicians to identify the cause of allergic reactions. Research methods such as western blotting, protein and peptide array testing, basophil activation, and flow cytometry are also used to evaluate immune responses to allergens or specific allergen proteins.

Identifying the specific allergen components that elicit an allergic immune response has been critical to improving diagnostics and therapeutics. Some extracts may have hundreds of proteins and a dozen or fewer allergens. Usually allergens are identified by performing two-dimensional electrophoresis and an anti-IgE detection method to visualize IgE reactive spots demonstrated by serum from allergic individuals. It is possible to identify hundreds of proteins when the gel is stained for protein. The number of allergens, proteins that specifically bind IgE, is a small proportion of all the spots.



Nomenclature of purified allergens includes the first 3 letters of the allergen genus, the first letter of the species, and a number that is assigned based on the order of discovery (e.g. peanut is *Arachis hypogaea* and one of its allergens is Ara h 1). Major allergens will bind IgE from over half of allergic patients' sera. The potency of some immunotherapy extracts is determined by the amount of a major allergen in the extract (e.g. Fel d 1 in cat extract). Although imperfect, it helps to reduce variability in immunotherapy dosing. Clearly, the use of several allergen proteins could better reduce variability and purified allergen proteins would eliminate the problem altogether.

Proteomics, the wide scale study of protein structure and function, has made it possible to identify increasingly smaller amounts of proteins. Proteomics has identified several allergens in honey bee venom that are in very low abundance in extracts and bind specific IgE from honey bee allergic individuals and beekeepers serum.[2] Such low abundance allergens may be very important to improve performance of diagnostic tests and therapeutic vaccines as natural or recombinant forms.

Some purified allergen proteins have been used to identify and measure the amount of specific IgE in patients clinically allergic to the extract. Serological studies of natural and recombinant allegens including peanut[3], honey bee[2], milk, kiwifruit[4], cherry[5], wheat[6], dust mite, grass, tree and weed[7] proteins have demonstrated IgE and some IgG_4 responses to allergens. Studies have shown that in some populations using purified allergens for serological testing is as sensitive as crude allergen extracts, and in others the crude extract remains more sensitive identifying clinical allergey.

Studies have ascertained that IgE reactivity to specific allergens may be more clinically relevant than others[3, 6]. Matsuo et al. demonstrated that specific IgE to a recombinant wheat protein, ω–5 gliadin, was much more sensitive identifying patients with wheat dependent exercise induced asthma than wheat or gluten. Nicolau et al. demonstrated that among 933 eight year old children from the UK, 110 (11.8%) were sensitized to peanut. Of those sensitized children, blood was drawn from 69 who were categorized either as peanut allergic, 17, or peanut tolerant, 52. In addition blood was drawn from 12 peanut allergic children to increase the peanut allergic sample size. The blood from all 81 children was tested in a microarray method that included three purified peanut allergens (Ara h 1, 2, 3, and 8) and several purified recombinant allergens that are cross-reactive with peanut extract. They included 5 grass allergens (Phl p 1, 4, 5b, 7 and 12) birch allergen (Bet v 1) and peach allergen (Pru p 3). In addition, the crossreactive carbohydrate determinant (CCD) was tested. Natural Ara h2 discriminated best between the peanut tolerant and allergic groups. Ara h 1 and Ara h 3 also associated with the peanut allergic groups, but were not nearly as discriminating as Ara h 2. Some of the recombinant grass allergens, Phl p 5b, 1, and 4 associated more with the peanut tolerant group, but again not nearly as strongly as Ara h 2 was associated with the peanut allergic group. Ara h 2 alone was almost as good at identifying peanut allergic children as a complex analysis of all the individual allergens together. These results from a cohort of 8 year old children in the UK suggest that one allergen may be more important when assessing clinical allergy.

A number of purified food allergens crossreact with pollen allergens and can have various degrees of clinical relevance depending on geography, genetics, and dietary customs[8]. Relevant allergens in one

population or location may be different in others. The complexity of exposure, cross-reactivity and genetics suggest that no single purified allergen will have global relevance. However, regionally individual allergens may be important for identifying individuals with clinical allergy , and lead to relevant immunotherapy within specific populations.

Allermetrix is currently developing a specific IgE test for the peanut allergen Ara h 2. Using Ara h 2 in a panel of allergens selected to identify cross reactions and true peanut reactions may help identify individuals truly allergic to peanuts. As demonstrated in the study by Nicolaou et al., in an open population of 8 year old children, about 12% are immunologically sensitized. However, only 22% of those sensitized had "true" peanut allergy. Making such panel testing available offers more information to clinicians with peanut sensitive patients and may identify true peanut allergic patients earlier that before.

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