In Vitro Methods for Diagnosing Allergy and Directing Therapy

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Abstract

Allergy and concomitant respiratory disease present an enormous and steadily growing healthcare burden. Early and accurate diagnosis is essential for best patient care. Knowledge of specific allergen sensitization enables physicians to administer appropriate immunotherapy and helps them to educate their patients in lifestyle changes that can halt progression of allergic disease. Several testing methods are available. Skin testing and food challenge are usually performed only by trained allergists, while *in vitro* blood testing can be requested by any physician. *In vitro* testing carries fewer risks and discomfort for the patient, and has been shown to be comparable in sensitivity and specificity to skin testing. Recombinant and/or highly purified molecular antigens have improved *in vitro* testing specificity, and provide greater insight into the molecular basis of allergic sensitization. Component-resolved testing can help identify exact antigens responsible for allergic sensitization, identifying patients at the greatest risk for severe reaction and/or anaphylaxis.

Keywords

Radioallergosorbent test (RAST), immunoassay, allergy, specific IgE, immunotherapy, skin testing, food challenge, allergen, allergy blood testing, cross-reactivity

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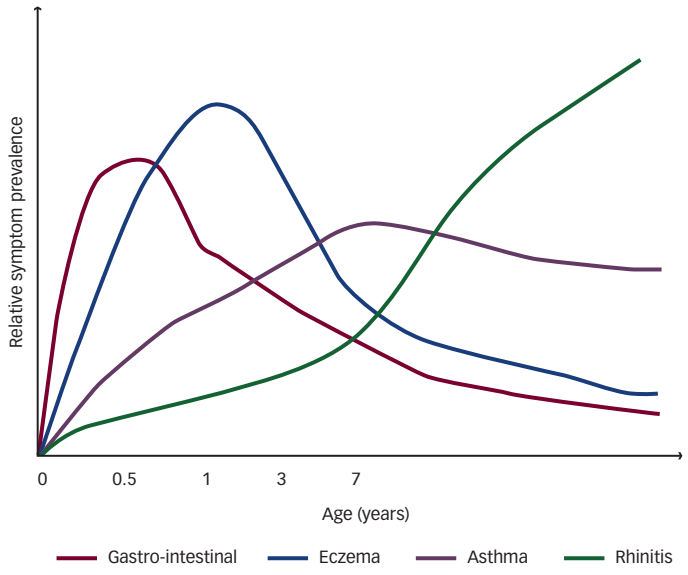
Respiratory illness related to allergy is a large and growing concern worldwide, especially in the US and other industrialized nations. Currently, allergic diseases affect approximately 40–50 million Americans, generating an enormous healthcare burden.[1] Annual US costs exceed $32billion, taking into consideration the combined costs of visits to healthcare providers, hospitalizations, and emergent care, medications, and lost employment time.[2]

Allergy can substantially reduce quality of life by imposing restrictions on outdoor activities, reducing athletic endurance and decreasing employment productivity. Time lost from school by the allergic child can negatively impact school performance and academic achievement, with long-lasting effects. Some allergies, such as peanut, tree nut allergy or cow's milk, can put a school-aged child at grave risk from accidental exposure.[3] The allergy burden is expected to rise in response to manmade and natural environmental factors contributing to reduced air quality and the increased outdoor pollen levels anticipated with climate change.[4] Airborne allergies are not the only triggers of respiratory symptoms, but food allergies, which most commonly first manifest with cutaneous, dermatologic or gastric symptoms, can also give rise to respiratory symptoms, ranging from mild to severe.

Allergy can arise at any age, but has been found to be most prevalent in young children. Allergic sensitization can manifest shortly after birth, often as atopic dermatitis or eczema. Several studies suggest that infantile colic is a manifestation of allergic sensitization to allergens in breast milk or formula.[5,6] Many children who are diagnosed with single or multiple allergies—usually to food—as babies or toddlers may also develop allergic disease or asthma related to airborne allergens, such as tree or grass pollen, animal dander, or dust mites some time between preschool and adolescence (see *Figure 1*). While allergy and early wheezing is more prevalent in boys in early childhood,[7] allergic rhinitis and asthma are more prevalent in girls by early adolescence.[8]

Several studies have shown that the early use of immunotherapy[9,10] or early use of antihistamines such as cetirizine (Zyrtec)[11] and ketotifen[12] may halt allergic progression and asthma onset (note that ketotifen is US Food and Drug Administration [FDA] approved in the US but only in an ophthalmic form). Allergen avoidance may also halt progression, as shown by the use of hypoallergenic bedding in the Study on the Prevention of Allergy in Children in Europe (SPACE) study, which led to a 4% reduction in dust mite, egg, and milk allergies in newborns at high risk for atopy,[13] as well as reduced sensitization in children between the five and seven years of age.[14] Thus, early recognition and accurate

Figure 1: Progressive Development of Allergic Disease



Adapted from Wahn.[15]

Table 1: Patient Conditions That May Preclude Puncture Skin Testing

Patient Type	Reason
Extensive skin disease	Can interfere with differentiating disease from wheal and flare reaction
Dermatographism	High levels of IgE in the skin can cause false positives; wheal and flare may be obscured
Use of long-acting antihistamines, tricyclic antidepressants, or other drugs that cannot be stopped for 2–11 days prior to testing[20]	Compounds limit response to allergens
A clinical history suggestive of anaphylaxis or life-threatening pharyngeal edema	Risk for systemic reaction
Pregnancy	Risk for systemic reaction

IgE = immunoglobulin E.

diagnosis followed by appropriate avoidance and/or therapy can be important for a long-term favorable outcome.

The Role of Sensitive and Specific Testing

While clinical history and examination are essential components of the allergy diagnostic process, American Academy of Allergy, Asthma and Immunology practice guidelines recommend testing by at least one of several methods for confirming the diagnosis, identifying causative agents and selecting appropriate therapy.[16–18] The most common methods used include puncture skin testing (PST), *in vitro* testing (IVT) or, in the case of food allergy, oral food challenge (OFC). The latter can be performed as an open challenge or a double-blind, placebo-controlled food challenge (DBPCFC).

Skin testing and food challenge are usually performed by certified allergists or immunologists in the office or appropriate care facility, and are well-established methods for diagnosing inhalation and/or food allergy. Both of these tests rely on an observable physiological response to suspected allergens applied to the skin or ingested. Although the sensitivity of these tests is generally good, and can be as high as 90%, both have limitations. The specificity of PST can be as low as 50%, especially when testing allergens to which the patient has shown no history of response. Both sensitivity and specificity can be hampered by the purity of the extract used: highly labile allergens may degrade during the extraction process or while in storage, affecting sensitivity.[19] Contaminants from other sources, such as pesticides or fungi in plant extracts, can also affect both sensitivity and specificity.[19] According to the 2008 Allergy Diagnosis Practice Parameters published by the Joint Council of Allergy, Asthma, and Immunology (JCAAI), several other factors may preclude or affect the usefulness of PST.[20] (see *Table 1*). Furthermore, interpretation of results is subjective and depends on the practitioner's experience and expertise.

Although food challenge is considered to be the gold-standard for food allergy diagnosis, it is usually only performed if PST or IVT is negative and suspicion remains high. It is not generally recommended for patients at risk for a severe or anaphylactic response and should be conducted in a facility that is appropriately equipped to handle potentially life-threatening reactions.[21] Even if there is some risk for anaphylaxis or life-threatening laryngeal edema, food challenge may still need to be considered in appropriate clinical situations. However, food challenge can present some logistical difficulties as it can take several hours or even days to administer if multiple allergens are suspected[22] and is also expensive. In addition, open food challenges can be influenced by the psychological response of the patient to a food.

In contrast, IVT evaluates the presence and level of specific IgE (sIgE) to suspected allergens. Requiring only a blood draw, there is no risk for evoking an allergic response in the patient and medication does not need to be halted. Testing can be requested by any type of physician, and IVT can serve as a good investigatory tool by primary care physicians to help determine or justify referral to specialists. Well beyond the proof-of-concept stage, commercial assays for sIgE detection and allergy diagnosis were first introduced in 1972. Early assays used radioactive markers to detect sIgE to allergens bound to a solid matrix (called a radioallergosorbent test [RAST]). Modern assays now use either fluorescence or chemiluminescence and have better speed, sensitivity, and specificity than their progenitors. As Hamilton and Williams point out, RAST is an outdated test and should no longer be used.[23]

Assays by the three primary manufactures (Siemens 3gAllergy, Phadia ImmunoCAP, and HYCOR HYTEC) have similar sensitivities and specificities for most antigens that have been compared. To date, all have demonstrated good performance and are routinely used to aid in allergy confirmation and the identification of primary causative agents, although false-negative results have been reported in some individuals. Several studies have demonstrated equivalent agreement between PST and IVT. In general, sensitivity of *in vitro* assays is similar to that of PST, and specificity is frequently greater than PST, depending on the extract type or process used to develop each specific allergen assay.[24–27]

Table 2: Allergen Databases

Database and Sponsor	URL	Utility
Allergome		
Allergy Data Laboratories, a non-for-profit company (ADL)	http://www.allergome.org	Sequences Cross-reactivity Clinical relevance Natural and recombinant form characteristics
Allergen Database		
Food Safety and Quality Directorate of The Food and Environment Research Agency at Sand Hutton York, UK	http://allergen.csl.gov.uk	Sequences Epitopes Homology
AllergenOnline		
University of Nebraska-Lincoln, The Food Allergy Research and Resource Program	http://www.allergenonline.com	Cross-reactivity Links to National Center for Biotechnology Information protein database
AllFam		
University of Vienna	http://www.meduniwien.ac.at/allergens/allfam	Protein family classification Based on Allergome database
Inform All		
European Union	http://foodallergens.ifr.ac.uk	General Clinical Biochemical Protein subunits Appropriate for the general population and practitioners
IUIS/WHO Allergen Nomenclature		
World Health Organization, International Union of Immunological Societies	http://www.allergen.org	Nomenclature organized by taxonomy
Structural Database of Allergen Proteins		
University of Texas Medical Branch	http://fermi.utmb.edu	Sequences 3D structures B-cell epitopes

IUIS = International Union of Immunological Societies; WHO = World Health Organization.

The Predictive Value of Specific Immunoglobulin E Quantification

PST and IVT generally have high negative-predictive values and can be good rule-out tests. However, both types of tests can have low specificity and poor positive predictive value if applied as general screening tests for allergens to which the patient is not already suspected of having primary sensitization. For the most part, allergens can be classified into specific classes of proteins. Many allergens within classes, such as profilins and lipid transfer proteins, can share substantial sequence homology across organisms and IgE directed to a true sensitizing allergen can cross-react with similar proteins. Even though the patient may have no clinically-significant reaction to a cross-reacting allergen, a false-positive response can be yielded by both PST and IVT. Several excellent review articles describe allergen families and cross-reactive capabilities[28–31] and multiple websites provide information on allergen classification and properties (see *Table 2*). Cross-reaction will be discussed further in the 'Future Directions' section of this article.

Several studies have attempted to define sIgE cut-offs predictive of clinical response. Sampson, for example,[32] determined that sIgE levels ranging from 2–20kU$_A$/L had positive predictive values ranging from 73–100% for the seven most common allergens in children (egg, cow's milk, peanut, fish, tree nuts, soybean, and wheat) using one system. Sampson's study was performed on an analyzer unable to detect IgE below 0.35kU$_A$/L and assumed that this level also represented the lower limit for clinically relevant sIgE levels.

Studies by Ollert et al.[25] demonstrated that this range could be extended to 0.10kU$_A$/L, conferring greater sensitivity, while Grunwald et al.determined that levels of sIgE below 0.35kU$_A$/L could evoke an allergic response.[33] Subsequently, other researchers and College of American Pathologists proficiency surveys have shown that systems may under- or over-report relative to each other because of differences in calibration and epitope selection. They have also shown that the cut-offs determined using one system cannot be used to interpret levels reported by other systems.[23–25,34]

Future Directions – Specific Antigens and Component-resolved Diagnosis

Allergy testing is only as good as the material used to capture sIgE. Until less than a decade ago, both PST and IVT were performed using extracts

from allergenic sources. Variability in extract quality can greatly affect results. Many allergens are heat labile or degrade easily and may not maintain their native conformation, which can decrease sensitivity. Specificity may be reduced by contaminants in the extract, such as pesticides or even fungi or bacteria growing on vegetables or pollens. Added to this, organisms are composed of thousands of different types of proteins and glycoproteins, only a fraction of which have been positively identified as being allergenic. Then too, an allergic individual may react to all or only some of the allergenic protein species.

Those allergenic proteins in a source recognized by more than 50% of allergic patients are termed major allergens. Minor allergenic proteins are of less importance and are recognized less frequently by sensitized patients.[35] The introduction of molecular biology techniques to the study of allergic disease has contributed greatly to the identification and understanding of these allergenic proteins. Based on cDNA sequencing and 3D structure analysis, most allergens have been grouped into a limited number of structural protein families independent of their biological sources. The structural similarities among the various proteins accounts for their subsequent immunological cross-reactions.[36]

Significant scientific advances and new perspectives in IVTs allows for detailed analysis of IgE specificity to the individual proteins that are responsible for the allergic response.[36] In order to diagnose allergy at the protein level, serum sIgE tests have been introduced using purified natural or recombinant single allergenic proteins or carbohydrate moieties.[36–38] Due to allergen cross-reactivity, patients may generate IgE antibodies to a particular sensitizing allergen; at the same time, a patient may demonstrate similar allergic reactions to many taxonomically or biochemically related allergens.

Allergen cross-reactivity occurs because IgE antibodies recognize antigenic determinants that are shared among a variety of allergenic sources (pollens, mites, molds, animal proteins, venoms and foods).[36,39,40] For example, in the case of pollen-food syndrome, patients sensitized to birch pollen frequently experience allergic symptoms upon first ingestion of other plant species (grains, vegetables, fruits, and nuts). These symptoms typically include itching, tingling, or swelling of the mouth and/or lips and are termed oral allergy syndrome (OAS). For many of these patients, IVTs using specific antigens rather than whole extracts can accurately measure IgE specific for the molecular allergenic proteins responsible for the oral allergy symptoms associated with pollen-food syndrome.

The best characterized allergen that has been found to be associated with pollen-food syndrome and OAS is Bet v 1, a member of the pathogenesis-related (PR-10) family of proteins. These molecules are heat labile and, as with other heat-labile allergens, generally evoke only mild, localized symptoms.[36]

More than 95% of birch-pollen-allergic patients have IgE reactive to Bet v 1; however, more than 60% are exclusively sensitized to the Bet v 1 molecule in birch pollen and not to other allergens contained in fruits and vegetables. Additionally, many patients with OAS have sIgE to PR-10 proteins that are highly homologous to Bet v 1, such as Pru av 1 from cherry and Mal d 1 from apple. Specific immunotherapy against Bet v1 based on this knowledge has been shown to reduce fruit-induced OAS.[41]

Profilins are highly cross-reactive allergens called pan-allergens. IgE from sensitized patients demonstrates extensive cross-reactivity with profilin from botanically unrelated plant sources (trees, weeds, grasses) and plant-derived food allergens (fruits, nuts, vegetables). Patients with IgE to profilin are either sensitized or at risk for developing allergic reactions to various plant pollens or plant-derived foods. Two examples of profilin allergens are Bet v 2, a minor allergen in birch pollen, and Pru av 4 from cherry. Like Bet v 1, IgE to profilins is associated with mild OAS symptoms.[36]

It is known that IgE reactivity to a specific protein can be associated with varying severity of symptoms. For example, IgE reactivity to PR-10 proteins such as Bet v 1 or to the pan-allergen profilin usually only evokes mild symptoms, such as local edema. However, the presence of IgE to allergens from the PR-14 family (lipid transfer proteins) can lead to severe systemic reactions, such as wheezing, vomiting, pharyngeal edema and anaphylaxis.[36,42–44] These proteins are generally resistant to degradation by heat or digestion. They are associated with food allergy not preceded by pollen sensitization. Major food allergens in this category include Pru p 3 (peach), Pru av 3 (cherry), and Ara h 9 (peanut).[36]

The use of molecular allergens in component-resolved diagnosis, where clustering of allergenic source reactivity is determined, rather than traditional testing using whole-extract allergens can aid in identification of the sensitizing agent and ultimately to improvement in the treatment of patients. As an example of this, Asarnoj et al.[45] found that Swedish children with peanut allergy preceded by birch allergy had sIgE to both Bet v 1 and Ara h 8, but had only mild reactions to peanuts. Since Ara h 8 is in the PR-10 family of proteins, it is likely that these children will never develop severe symptoms to peanuts and may not need to practice strict avoidance.

On the other hand, children reactive to Ara h 2 experienced significant reactions to peanuts. Those who were sensitized to Ara h 2 and either Ara h 1, Ara h 3, or both Ara h 1 and h 3, had the worst symptoms and were at the greatest risk for anaphylaxis (Ara h 9 has also been shown to confer similar risk in Mediteranean populations).[45] Since Ara h 2 shares common epitopes with almonds and brazil nuts, this can explain why some children with a peanut allergy are also sensitive to these tree nuts, while children who are not sensitized to Ara h 2 are not.[28]

Conclusion

Since the primary care physician or pediatrician is likely to be the first practitioner to evaluate possible allergy, IVT provides a convenient, reproducible and reliable tool that does not require extensive training for interpretation. Component testing is likely to contribute to better specificity with respect to identifying the exact allergens responsible for clinical reactions, although it may offer more complexity in interpretation. Once the likelihood of allergy has been established, the primary care practitioner can determine whether referral to an allergist is appropriate for further testing, refinement of diagnosis and specific immunotherapy.[46] ■

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