Hot Topic

Hypersensitivity Reactions and Peanut Component Testing
Hello everyone. My name is Melissa Snyder, and I am the director of the Antibody Immunology Laboratory at Mayo Clinic in Rochester, Minnesota. I'm so glad you are able to join me for a brief discussion about the diagnosis of allergic disease and the role of component allergen testing.
Disclosures

• None

Related to the content of this presentation, I have no financial disclosures.
Utilization management is becoming increasingly relevant for most medical institutions. While viewing this presentation, take some time to consider a few points, including how this testing might be used in your practice? When the tests should be ordered? and How the results from this testing might impact patient management.
Allergic disease is caused by a pathologic immune response, which results from an uncontrolled immune reaction against an inappropriate antigen, which are commonly referred to as “allergens”. Allergens are considered to be “inappropriate antigens” because they are normally inert molecules that, for most individuals, do not activate any part of the immune response. However, in a person with allergic disease, exposure to an allergen leads to development of a type I hypersensitivity reaction. Type I reactions are also referred to as immediate hypersensitivity reaction because the pathological and clinical manifestations can begin quickly – sometimes only 2-3 minutes after allergen exposure. A type I hypersensitivity reaction is mediated by IgE, a specific class of immunoglobulin, in conjunction with mast cells.
IgE is the least abundant immunoglobulin in circulation, accounting for only 0.02 to 0.05% of total immunoglobulins. This puts the concentration range for IgE on the order of 20-200 ng/mL in adults. Also, IgE has the shortest half-life of all the immunoglobulins at 2 days, compared to IgG, for example, which has a half-life of close to 20 days. The physiological role of IgE in adaptive immunity is not well-understood. However, it is thought to be important in the immune response to infections by helminths and protozoan parasites. Mast cells are a type of granulocyte which, as the name implies, contain many cytoplasmic granules. These granules contain a variety of inflammatory mediators, including tryptase and histamine. Mast cells are predominantly tissue-resident cells, and are found to high densities in the skin and gastrointestinal system. Mast cells are linked to IgE through expression of a high-affinity receptor specific for this immunoglobulin class.
In a genetically susceptible individual, exposure to a normally non-immunogenic molecule will result in a type I hypersensitivity reaction. The progression of allergic disease from this reaction can be divided into 3 phases. In the sensitization phase, an individual will produce an IgE antibody that is specific for a given allergen. At this point, due to the presence of an allergen-specific IgE, we refer to this individual as being “sensitized”. Most IgE that is produced during the sensitization phase will bind to the high-affinity IgE receptor that is present on the mast cells. At this stage, the mast cells are primed for activation, which leads into the activation phase. During the activation phase, re-exposure to the allergen leads to mast cell activation through cross-linking of the IgE receptor. Activation of the mast cells leads quickly to the effector phase, in which the inflammatory mediators are released from the granules within the mast cells. It is this step that ultimately results in the clinical manifestations associated with an allergic response.
It is estimated that nearly 50 million Americans suffer from allergic disease in one form or another. Among all ages, allergy is the 5th most common chronic disease. However, in children, it is the 3rd most common chronic disease. You are probably familiar with many of the common clinical manifestations associated with allergy, which include rhinitis, dermatitis and eczema. In addition, some individuals, especially small children, may display with symptoms that predominantly affect the gastrointestinal system. Anaphylaxis, although relatively rare, is a serious consequence of allergic disease. Anaphylaxis is a life-threatening systemic hypersensitivity reaction that results from large-scale mast cell activation. Without immediate treatment, there is a serious risk of significant morbidity and mortality associated with an anaphylactic episode. Individuals may be allergic to a large variety of molecules. These different allergens can be grouped into classes, including foods, pollens, insects, drugs, dust, animals, molds, and even occupational chemicals. The wide variety of allergens makes testing for the potential cause of an allergy quite challenging.
In establishing a diagnosis of allergic disease, demonstrating a compatible clinical history is critical. Specifically, it is important to show that a correlation exists between exposure to a potential allergen and development of compatible clinical symptoms. In addition, a detailed family history is necessary. Given the genetic component, it is not uncommon to find that allergic disease occurs in multiple family members. Once allergic disease is suspected based on the clinical history, identification of the allergen-specific IgE is usually pursued. This can be accomplished either through in vivo skin testing or in vitro allergen-specific IgE testing. For most patients, a compatible clinical history in conjunction with IgE testing is usually sufficient to establish an allergy diagnosis. However, for a small subset of patients, demonstration that exposure to a candidate allergen actually results in clinical disease may be needed. This is generally performed as either a food or an inhalant challenge, such as in the context of a potential pollen allergy. This type of testing must be performed by a physician in a controlled environment, due to the risk for potential serious reactions. For the purposes of our talk today, we will focus on the testing used for the identification of the allergen-specific IgE.
We will begin with skin testing. Skin testing is an in vivo bioassay, which measures the effects of mast cell activation that occurs after intradermal injection of a candidate allergen. If an individual has an allergen-specific IgE, the injected allergen will bind to IgE found on mast cells present in the skin. The binding of the allergen to the IgE leads to mast cell activation, release of histamine and tryptase, and the classic "wheal and flare" reaction. There are two forms of testing — the skin prick or skin puncture test and the intracutaneous test. The skin prick tests are more common, due to general lack of specificity of the intracutaneous tests. With skin testing, it is important to remember that a positive response only identifies an individual as "sensitized". In other words, this person has an IgE antibody that is recognizing a specific allergen. It is not diagnostic for allergic disease.
The other option available is to perform in vitro allergen-specific IgE testing, which is most commonly performed by sandwich immunoassay. In this testing, the patient sample is added to an immobilized allergen. Any antibody specific for that allergen will bind to the immobilized reagent; detection then occurs through the use of a labeled anti-IgE antibody. This approach is often referred to as RAST testing. RAST stands for “radioallergosorbent test” and is a reference to the historical method in which radiolabeled anti-IgE was used for detection. Although radiolabeled reagents are no longer used, the RAST terminology has persisted. Again, we must remember that, similar to skin testing, the detection of an allergen-specific IgE by this methodology only identifies a sensitized individual and is not necessarily diagnostic for allergic disease.
At this point, we should probably understand what the difference is between “sensitization” and “clinical allergy”. In sensitization, an individual may have an IgE antibody that will bind to a specific molecule, although they manifest no clinical symptoms when naturally exposed to that allergen. In contrast, an individual with a true allergy will develop clinical symptoms when exposed to a given allergen, which would be mediated by the presence of a specific IgE antibody. The next question that might come to mind is how can we have sensitization in the absence of allergic disease? The absence of clinical allergy may be due to differences in the environmental exposure to the allergen, including the route of exposure, the duration of the exposure, or how much allergen the individual is exposed to at a given time. In addition, coexisting conditions that the individual might have could also have some effect. Also, it is possible that a person could be sensitized to a protein that has very little or no allergenic potential. For example, a person could be sensitized to a food protein that is degraded during the digestion process. In this case, the individual might have a positive skin or in vitro test, demonstrating that they have an allergen-specific IgE for this protein. However, when the food is ingested, the protein is degraded such that it cannot bind to the IgE antibodies and thereby does not initiate an allergic response.
It is for these reasons – differences in environmental exposure and sensitization to nonallergenic proteins – that the presence of an allergen-specific IgE alone is not sufficient to diagnose allergic disease. However, in a person with a compatible clinical history, identification of the relevant allergen-specific IgE can provide additional information that is relevant for diagnosis and, in some cases, treatment decisions. Focusing on the in vitro testing options, we have the allergen-specific IgE testing and the component IgE testing. In allergen-specific IgE testing, an extract from the biological material, such as an organism, cell, plant, or food, is used as the capture allergen. This extract is a complex mixture of proteins, and likely would include both allergenic and nonallergenic molecules. In comparison, component IgE testing uses individual proteins, or components, from the biological extract as the capture antigen. Generally, these are either purified or recombinant proteins that have been identified as molecules associated with clinical allergic disease.
To understand this difference more, we can use in vitro testing for peanut sensitization as an example. In the “Peanut, IgE” testing, an extract from total peanut is used as the capture antigen. Peanuts are relatively complex and contain over 30 proteins, only about half of which have been associated with sensitization.

In contrast, in “Peanut component, IgE” testing, 5 specific peanut proteins are used as the capture antigens, namely Ara h 1, Ara h 2, Ara h 3, Ara h 8, and Ara h 9. Instead of assessing for an IgE that might recognize an unknown protein present within the peanut extract, using this approach we are identifying an IgE that is specific for a well-defined protein component.

### Laboratory Testing: Extract and Component IgE

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<th>Peanut, IgE</th>
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<tr>
<td>Mixture of proteins prepared from extraction of mature peanuts</td>
<td>Ara h 1</td>
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<td>Peanuts contain over 30 proteins, 18 of which associated with sensitization</td>
<td>Ara h 2</td>
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<td>Ara h 9</td>
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These different peanut component proteins have different properties and functions, and can be divided either into seed storage or nonstorage proteins. Ara h 1, 2, and 3 are all seed storage proteins, although they belong to different protein families. Ara h 1, also referred to as vicilin, is a member of the 7S globulin family. Ara h 2, also known as conglutin, is part of the 2S albumin family. Ara h 3, also referred to as glycine, is part of the 11S globulin family. In the nonstorage category, we have Ara h 8 and 9. Ara h 8 is a member of the PR-10 protein family and is a Bet v 1-homologue; Ara h 9 is a lipid transfer protein. Each of these 5 proteins has been identified as being a potential target for allergen-specific IgE antibodies. In the next few slides, we will begin to understand how testing for peanut component IgE antibodies can be used for the evaluation of patients with suspected peanut allergy.
The most obvious use of peanut component IgE testing is as a diagnostic aid for peanut allergy. Most studies have demonstrated that peanut component IgE testing offers significant diagnostic improvement over total peanut IgE testing. In this example, a ROC analysis is shown which compares the diagnostic utility of peanut skin prick testing, total peanut IgE, and Ara h 2 testing for diagnosis of true allergic response as assessed through an oral food challenge. The blue line represents total peanut IgE, which, among the 3 tests, shows the poorest correlation with clinical peanut allergy. In contrast, Ara h 2 IgE testing is comparable to the diagnostic performance of skin prick testing. This finding has been confirmed in multiple studies, each of which demonstrate that Ara h 2 IgE has the highest predictive value for peanut allergy and offers improved diagnostic utility over total peanut IgE.
In addition to diagnostic implications, peanut component testing may be useful for assessing the risk of severe allergic responses. The presence of IgE antibodies against Ara h 1, 2, or 3 has been associated with a risk of systemic allergic reactions. This is related to certain characteristics of these seed storage proteins, namely that their allergenic potential is not affected, or may even be increased, by heating or processing of the peanuts and that they are relatively resistant to proteolytic degradation. In comparison, Ara h 8 and 9 IgE antibodies seem to correlate more with localized and limited allergic responses. In fact, both of these antibodies are associated with significant cross-reactivity, which brings us to the concept of oral allergy syndrome.

Laboratory Testing: Prognostic Application of Component IgEs

- Predicting severity of clinical allergic response
Oral allergy syndrome is a class of food allergy in which the allergic reaction is restricted to the mouth, generally after eating certain fruits, nuts, or vegetables. This phenomenon often occurs in persons with known pollen allergy. In this case, the food allergy is associated with cross-reactivity of the allergen-specific IgE. Ara h 8, as mentioned before, is a homologue of Bet v 1, which is a primary birch pollen allergen. Patients with birch allergy who are Ara h 8 positive may have localized peanut reactions due to this cross-reactivity. Similarly, Ara h 9 IgE antibodies target a lipid transfer protein, which is relatively non-specific. Positivity for Ara h 9 IgE has been associated with cross-reactivity in patients with pitted fruit allergies.
As we can see, there are multiple advantages of testing for peanut component IgEs. There is improved diagnostic utility for evaluation of patients with suspected peanut allergy, especially for Ara h 2 IgE antibodies. Peanut component IgE testing can be used prognostically, as Ara h 1, 2, and 3 IgE antibodies are more frequently associated with systemic clinical reactions. And lastly, peanut component IgE testing can be used to distinguish between true peanut allergy and cross-reactivity, through the use of Ara h 8 and 9 IgE antibodies.
Recognizing that peanut component IgE testing may be useful for some patients, while being cognizant of managing the utilization of allergy testing, Mayo Medical Laboratories has incorporated peanut component IgE testing within a reflex algorithm. When “Peanut, IgE with Reflex to Peanut Components, IgE, Serum (PEANT)” is ordered, all samples are initially tested using the total peanut IgE assay. If this result is negative, or less than 0.1 kU/L, no further testing is done, since sensitization to peanut allergens was not detected. In contrast, if the total peanut is positive, the sample is automatically reflexed to the 5 component IgE tests. It is important to perform all of the component tests, as this informs to a better overall interpretation. The goal of this algorithm is to target the peanut component testing to the most appropriate patients, and serves as an excellent example of on-going efforts to help clinicians and hospitals manage test utilization.
To summarize, we must remember that allergen-specific IgE testing only identifies sensitized individuals, and that the link between sensitization and allergy is not always clear. With this caveat, identification of allergen-specific IgEs, either through skin or in vitro testing, remains an important part of the evaluation of patients with suspected allergy. Component IgE testing may provide additional information, specifically as an adjunct to extract-based allergen-specific IgE testing. At this time, the primary utility of component testing is for peanut allergy. However, the use and availability of component testing is likely to expand as we learn more about specific proteins associated with allergic diseases.
Thank you for joining me for this Hot Topic on peanut component testing.

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