

**TITLE: Allergy Testing for Allergic Rhinitis**

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**RESIDENT PHYSICIAN: Michael Briscoe Jr., MD**

**FACULTY PHYSICIAN: Jing Shen, MD**

**SERIES EDITORS: Francis B. Quinn, Jr., MD**

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## **Introduction**

Allergic rhinitis affects about 1/3 of the US population. Morbidity from this disease leads to decreased productivity, lost work/school days, and increasing costs of medical care and treatment. It is defined as inflammation of the nasal mucosal lining caused by an exaggerated IgE mediated hypersensitivity to aeroallergens. Symptoms include rhinorrhea, nasal congestion, post nasal drip, sneezing, cough, itchy nose and eyes, and fatigue. It is an important entity for the practicing otolaryngologist because many of these patients have failed medical management. In order to treat these patients, allergy testing may need to be performed in order to start vaccine immunotherapy.

Allergy testing has been around for many years. In 1872 pollen was identified as the causative factor for fall hay fever, and Blakely performed first skin test with pollen extract. In 1912, the first intradermal test was performed by Schloss. In the 1920's skin prick testing was introduced by Lewis and Grant. 1935 Hansel began using serial dilution testing (1:5 dilutions with endpoint testing) and Rinkel perfected serial endpoint testing in the 1940's. Recently, Krouse introduced modified quantitative testing. In order to understand the need for allergy testing, a brief review of immunology and how it affects allergic rhinitis is needed.

## **Review of Immunology in Allergic Disease:**

Allergy represents an exaggerated immunologic response to an otherwise innocuous agent, which causes harm to the host. The inciting agent is known as the allergen. There are four types of hypersensitivity reactions, which were originally characterized by Gell and Combs.

**Type I:** Immediate IgE mediated hypersensitivity causes rapid degranulation of mast cells with pro-inflammatory cytokines. IgE binds to mast cells via a high affinity Fc receptor. Characterized by early phase, within minutes, and late phase, hours after initial response. Examples include allergic rhinitis, food allergy, and allergic or atopic asthma.

**Type II:** Antibody mediated, in which antibodies bind to cells and causes damage or impairment of function. Examples include transfusion reactions, hemolytic anemias, hyperacute graft rejection Myasthenia Gravis and Goodpasture's syndrome.

**Type III:** Immune complex mediated occurs when IgG or IgM binds with antigens, and the complexes are deposited in tissues, especially small vessels. Once in the tissues, damage occurs secondary to complement activation. Examples include serum sickness, glomerulonephritis, and arthritis.

**Type IV:** T-cell mediated (delayed hypersensitivity), on first exposure, T cell is sensitized. On subsequent exposures, the allergen is detected on the surface of target cells and these cells are lysed by T cells. Examples include contact dermatitis, granulomatous diseases.

Allergic diseases important to the otolaryngologist are allergic rhinitis and food allergy, both of these are IgE mediated (type I). Early phase ranges from a minimal wheal and flare reaction to anaphylaxis. The response is characterized by vasodilation, vascular leakage, smooth muscle spasm and glandular secretions. These changes occur within 5 to 30 minutes and tend to subside within 60 minutes. Late phase reactions occur 2 to 8 hours after initial exposure and last for several days. Migration of eosinophils, neutrophils, basophils, and CD4+ T cells occurs and mucosal tissue damage also occurs.

## Cells Important for Allergic Response

**B cells** are the only lymphocytes that can produce antibodies. They mature in the bone marrow, and are responsible for humoral immunity. They produce IgA, IgD, IgE, IgG and IgM antibodies. IgA is a dimer that is predominantly found in secretions. IgD is produced by naïve B cells, and may be involved in antigen-induced lymphocyte proliferation. IgE is found in immediate hypersensitivity and helminthic infections. IgG is the major antibody of secondary responses. It is active against viruses, bacteria, and fungi, the only immunoglobulin that crosses the placenta, and fixes complement by the classic pathway. IgM is a pentamer and the predominant antibody in the early immune response. Naïve B cells produce IgM and IgD, and undergo isotype class switching under the influence of T cells (T<sub>H2</sub>) and certain antigens.

**T cells** travel from the bone marrow and mature in the thymus. They recognize peptide fragments of foreign proteins bound to self-major histocompatibility complex (MHC) in other cells in the body. T helper cells (CD4+) recognize antigens found on MHC class II molecules on antigen presenting cells. TH1 cells are involved in phagocyte mediated defenses against intracellular microbial infections. TH2 cells secrete IL-4, IL-5, IL-9, IL-10, and IL-13. TH2 cells down regulate TH1 cells, and induce B cell isotype switching. Catalytic T lymphocytes (CD8+) recognize antigens on MHC I molecules.

**Antigen presenting cells** include monocytes, macrophages, dendritic cells, and B cells. Process antigens and present peptides on their cell surface via MHC molecules that activate T cells.

**Mast Cells and Basophils** are the major effector of type I mediated hypersensitivity. IgE cross-links these cells causing rapid degranulation of their contents. Activation of these cells

leads to release of chemokines by three different pathways. 1) immediate release of histamine, heparin, proteases, and TNF alpha. This leads to vasodilatation and leaky vessels, as well as changes in the endothelium that allows migration other inflammatory cells. 2) enzymatic modification of arachidonic acid into prostaglandins and leukotrienes, within 1 or 2 hours. 3) Synthesis and secretion of IL-3, IL-4, IL-5, and GM-CSF, which recruit other inflammatory cells and are responsible for the late phase of an allergy attack.

## **IgE Mediated hypersensitivity**

The pathogenesis of a type I hypersensitivity reaction starts with IgE antibody production, also called the sensitization phase. Antigen is presented by antigen presenting cells to CD4+ Th2 cells. The activated Th2 cells then produces a cluster of cytokines, including IL-3, IL4, IL-5, IL-13 and GM-CSF. IL-4 is absolutely essential for turning on the IgE –producing B cells and for sustaining the development of Th2 cells. IL-3 and IL-5 promote the survival of eosinophils. IgE antibodies produced by B cells quickly attach to mast cells and basophils. When mast cells and basophils are exposed to antigen again, antigen binds to the IgE antibodies on the surface of these cells. Multivalent antigen causes cross-linking of IgE antibodies, which activates cell degranulation with discharge of preformed mediators and de novo synthesis of mediators. These mediators are responsible for the observed increased vascular permeability, increased mucus secretion, and smooth muscle contraction in the allergic reaction. These mediators also have chemotactic properties. Eosinophils, neutrophils, and monocytes are recruited and release additional waves of mediators. The recruited cells amplify and sustain the inflammatory response without additional exposure to the triggering antigen. This is the late phase reaction.

## **Allergic Rhinitis**

Inflammation of the membrane lining the nose secondary to hypersensitivity to aeroallergens, characterized by rhinorrhea, sneezing, pruritis, congestion, post nasal drip and associated conjunctival, otologic or pharyngeal inflammation. These symptoms can be episodic, seasonal or perennial. Severity ranges from mild, to seriously debilitating with excess days of missed school or work. Risk factors include family history of atopy, serum IgE > 100 IU/ml before age six, higher socioeconomic class, exposure to aeroallergens, presence of positive allergy skin prick test.

## **Differential Diagnosis**

- I. Infectious
- II. NARES (Nonallergic rhinitis with eosinophilia)
- III. Vasomotor rhinitis
- IV. Other rhinitis syndromes
  - a. Ciliary dyskinesia
  - b. Exercise induced
  - c. Atrophic rhinitis
  - d. Hormonally induced
    - i. Hypothyroidism
    - ii. Pregnancy

- iii. Oral contraceptives
    - iv. menstrual
  - e. Drug induced
    - i. Rhinitis medicamentosa
    - ii. Antihypertensive therapy
    - iii. Aspirin
    - iv. NSAIDs
  - f. Reflex induced
    - i. Gustatory
    - ii. Chemical or irritant induced
    - iii. Posture reflexes
    - iv. Nasal cycle
    - v. Emotional factors
  - g. Occupational
- V. Structural/mechanical
  - a. Deviated septum
  - b. Turbinate hypertrophy
  - c. Adenoid hypertrophy
  - d. Foreign body
  - e. Choanal atresia
  - f. Nasal tumors
- VI. Inflammatory
  - a. Wegener's granulomatosis
  - b. Sarcoidosis
  - c. Midline granuloma
  - d. Sjogren's syndrome
  - e. SLE
  - f. Nasal polyposis
- VII. Cerebrospinal fluid leak

## History and Physical Exam

It is important to illicit timing, severity, onset, duration, and effect on daily living. Many patients will have an idea of what triggers their symptoms and the seasonality of symptoms. Environmental questions should include home, work school/daycare exposures, and exposure to tobacco. Past nasal trauma, positive family history, current and past treatments, should all be included in history.

Physical exam includes a complete head and neck exam. Special attention is paid to the patient's general appearance. Facial pallor, allergic shiners, nasal crease, mouth breathing, and clubbing of the fingers can signify allergic rhinitis. Examine the eyes for conjunctivitis and Dennie-Morgan lines, accentuated lines or folds below the margin of the inferior eyelid. The nose may reveal polyps, enlarged turbinates, presence of mucus or purulent drainage, septal deviation or blood. The exam of the oropharynx may reveal tonsillar hypertrophy or cobblestoning. The ears must be examined for abnormalities to the middle ear, or tympanic membrane. The neck should be examined for lymphadenopathy and thyroid enlargement.

Auscultation of the lungs is necessary to assess for wheezing, or other signs of asthma, and the skin should be examined for eczema, dryness, or dermatographism.

## Pathophysiology of Allergic Rhinitis

Atopic subjects inherit the propensity to produce IgE-mast cell lymphocyte immune responses. Exposure to low levels of aeroallergens for prolonged periods of time leads to presentation of epitopes being presented to CD4+ cells by APC's. These CD4+ cells then secrete IL-3, IL-4, IL-5, GM-CSF and other cytokines. This promotes proliferation of plasma cells that produce IgE, mast cells, and infiltration of nasal mucosa and eosinophilia.

**Early response** with continued exposure, IgE coated mast cells infiltrate the nasal mucosa, and are activated when they encounter the allergen. Mast cells release, histamine, heparin, tryptase, kinase, chymase and other chemokines. Arachidonic acid is broken down to prostaglandins and leukotrienes that stimulate leaky vessels and nasal edema, release of mucus, and dilate arteriole-venule anastomoses causing occlusion of nasal air passages. Sensory nerves are stimulated and relay sensations of nasal itching and congestion, and initiate the sneeze reflex.

**Late response** occurs 2 to 11 hours after initial exposure. Mast cell chemokines affect the endothelium promoting VCAM and E-selectin expression. These molecules allow circulating leukocytes to stick to the endothelium. IL-5 attracts eosinophils, neutrophils, basophils, T cells, and macrophages. Over the course of 4 to 6 hours, these cells release even more chemokines. Eosinophils release major basic protein, eosinophil cationic protein, hypochlorate, and leukotrienes, which cause inflammation and damage seen in chronic allergic reactions.

## Allergy Testing

**Screening tests** should have the following characteristics: 1) be rapid, efficient, and cost effective method to assess allergy. 2) Antigens should be representative of what the patient may encounter, and should be geographically based. Most allergic individuals will react to common antigens via *in vivo* or *in vitro* techniques. Negative result usually requires no additional testing. Positive result requires further testing of other antigens in the group or family. There may be some cross-reactivity, especially with molds. Also, they should test for 12 to 14 antigens, (pollen, mold, weeds, dust mite, animal dander)

**Nasal smear** used to differentiate allergic rhinitis and NARES, from other forms of rhinitis. Typically find eosinophilia, but its absence does not rule out allergic rhinitis. May find neutrophils in smear as well.

**Skin testing** is the most widely used form of allergy testing. 2003 AAOA guidelines for allergy testing state:

- The goal of testing is to identify antigens to which patients are symptomatically reactive and to quantify the sensitivity if immunotherapy is planned
- There are a variety of acceptable techniques:
  - Prick testing, intradermal testing, intradermal dilutional testing, and in vitro

testing

- Allergy care shall be directed by a trained and competent physician who regularly participates in the care
- Members shall practice in an ethical and fiscally responsible manner

**Prick/scratch testing (SPT)** is a superficial skin reaction that does not penetrate dermis. It is highly specific, sensitive, convenient and safe. It does require a positive (histamine) and negative (saline) control. Disadvantages include: patient discomfort, intertester variability, and nonstandardized allergen extracts, as well as different interpretation scales. An example of this is the multitest II. This introduces 6 to 10 antigens plus the positive and negative control using an instrument that scratches the skin. A test is positive if there is a wheal and flare reaction which is greater than or equal to the histamine control.

**Intradermal testing (IT)** a dilute antigen extract is injected into the dermis, and a superficial wheal forms. After ten minutes, the wheal is measured again to see if there was any progression. If the diameter of the wheal has increased by 2mm or greater, then a positive response has occurred. This causes relatively minimal patient discomfort. Disadvantages include higher risk of anaphylaxis, time intensive and possible false positive.

**Intradermal dilutional testing/Set endpoint titration (IDT/SET)** Intradermal testing utilizing serial dilutions to quantify degree of sensitivity to specific antigen. Very labor intensive and uncomfortable to patient due to multiple sticks. Wheal measures similar to intradermal testing. 1<sup>st</sup> dilution that causes a wheal of 2mm, with progression of this wheal by another 2mm (confirmatory wheal). This type of testing is important for determining the initial concentration used for immunotherapy.

**Modified quantitative testing (MQT)** a hybrid of skin prick and IDT. Skin prick determines an approximate range of sensitivity, followed by a single intradermal test to further identify the level of sensitivity and quantify the allergic response.

**In Vitro testing RAST** (radioallergosorbent testing) RAST is a radioimmunoassay test developed in the late 60's for the detection of specific serum IgE antibodies. Initial studies demonstrated a 96% efficiency, sensitivity and specificity. The modified RAST is the form now used, introduced by Fadal and Nalebuff in 1977 with the advantages of increased test sensitivity without a loss in specificity.

Soluble allergens bound to solid phase support (paper disc) to create a stable immunosorbent media. The paper disc is incubated with the test sera, specific IgE antibody will bind to the solid phase allergen. The paper disc is then washed to remove all unbound sera and IgE. The disc is then exposed to rabbit anti-human IgE antibodies which are radiolabeled. It interacts with the Fc determinant portion on human IgE bound to the solid phase allergen. The unbound anti-IgE is washed off the disc and the disc is then quantified by a scintillation counter.

This test should be used when there are contraindications to skin testing. These include children that can not tolerate skin testing, patients on antihistamines, patients with dermatographism, and those taking beta blockers (may be impossible to treat anaphylaxis).

## Comparing the tests

### Efficacy

Gungor et al found that skin prick testing correlates with RAST and SET 81-89% of the time depending on the antigen. Skin prick testing is fast, inexpensive, and has only mild patient discomfort. However, there are false negatives, and this type of testing cannot be performed in patients who are on antihistamines.

Simons et al compared Multitest II (skin prick) to IDT. Found that patients were positive to more antigens with IDT. This may be because IDT is more sensitive or there could be more false positives. However, he did find that multitest II did correlate with the IDT endpoint. In 2006 McKay performed a retrospective chart review of patients with a positive IDT after negative skin prick test. Certain antigens were more likely to have a positive IDT after negative skin prick (dust mite, cockroach, fulsarium rough marsh elder, and ragweed). He concluded that this could be from glycerin reaction, or true positive due to the increased sensitivity of IDT. In order to know for sure, he recommended nasal provocation testing.

2007 Peltier et al performed a prospective study using five antigens to compare MQT, SPT, and IDT. Found a 77% concordance rate between MQT and IDT, wheal size from SPT is predictive of IDT endpoint, and that MQT is nearly as effective as IDT for starting doses of immunotherapy.

### Cost

2003 Shah et al compared multitesting with SET (MQT) versus IDT/SET. Concluded that multitesting is a cost effective screening test. MQT can be used to find the starting doses for immunotherapy and is one third less costly and time consuming than IDT/SET alone.

2006 Seshul et al defends the use of IDT/SET on an overall cost effectiveness. With IDT, the highest dose to safely start immunotherapy is known. Thus this is the starting dose for immunotherapy. With SPT, he found that it correlated poorly to endpoint titration. This would cause a lower starting dose, with more time and cost to reach the maintenance doses needed for successful immunotherapy.

## Adjuvant testing

**Nasal Endoscopy** allows direct visualization of nasal mucosa. It allows for accurate, site specific nasal smears and is important in ruling out other nasal pathology.

**Acoustic rhinometry** measures cross sectional area and intranasal cavity volume by bouncing sound signals on the nasal structures. Measurements are taken before and after decongestion. Nasal provocation takes this one step further, by introducing an allergen via a metered dose spray, then taking measurements. Cross sectional area 2, corresponds with the anterior border of the inferior turbinate, is the best site for assessing sensitivity to an allergen (Uzzamann et al). This is still an experimental test, but some Allergists have pushed for its use in the evaluation of allergic rhinitis. Dykewicz et al 1998 Joint Task Force on Practice

parameters in Allergy, Asthma and Immunology cited that nasal provocation has a role in workplace allergies. Also, it may play a role in deciphering whether IDT has higher sensitivity or higher rate of false positives than SPT.

## Treatment

**Environmental** measures should be taken by all people that suffer from allergic rhinitis. This includes avoidance of specific allergen, dehumidifiers, HEPA filters, special linens, weekly laundering of linens in hot water, frequent cleaning of household furniture, and minimizing carpet. For those with pets, they can consider removing pet from the home, the bedroom, and should wash the pet weekly.

**Medical** treatment is the mainstay of therapy for allergic rhinitis. There are many different classes of medications that can be used alone or in combination.

*Nasal saline* is an inexpensive treatment that is believed to cleanse the nasal mucosa of allergens.

*Mast cell stabilizers* such as cromolyn sulfate act by decreasing the release of mast cell contents. It is fairly safe, but must be used four times per day and can not be used as a rescue medication.

*Decongestants* work by vasoconstriction, which leads to decreased edema and increased nasal patency. Topical therapies such as oxymetazoline and phenylephrine provide quick relief, but can lead to tachyphylaxis and rhinitis medicamentosa with prolonged use. Oral medicines such as pseudoephedrine are also useful, but many over the counter medications are using it less often due to its significant side effects. These side effects include hypertension, tachyarrhythmia, wakefulness, and urinary retention.

*Antihistamines* decrease symptoms of sneezing, itching, and edema by blocking the H1 receptor. Diphenhydramine (benadryl) is the most well known drug in this class. It has H1 receptor blockade, peripheral and central, as well as anticholinergic effects. Its central H1 activity causes sedation, and its anticholinergic effects include dry oral/nasal mucosa, urinary retention, memory impairment, and blurred vision. Due to its side effect profile, second generation antihistamines were developed. Cetirizine, loratidine, desloratidine, and fexofenadine are oral preparations, and azelastine is topical. These are also known as non-sedating antihistamines, and are free of anticholinergic effects.

*Leukotriene receptor antagonists* block the late phase of the allergic response. Montelukast is approved for seasonal allergic rhinitis and is useful in abating sneezing and nasal congestion. Higher efficacy in patients with Samter's triad because of their increased production of leukotrienes.

*Intranasal steroids* should be first line therapy for allergic rhinitis. All of the drugs used in the US for allergic rhinitis have a good safety profile, and high efficacy, when used regularly. Many people prefer oral to intranasal medications, or cannot tolerate associated epistaxis or dry

mucosa. Also, there are concerns about growth suppression in children, and decreased bone density.

**Immunotherapy** should be considered for patients with evidence of specific IgE antibodies to clinically relevant allergens. It is an effective treatment for allergic rhinitis, asthma, and hymenoptera stings. The decision to begin immunotherapy depends on the severity of symptoms, and their resistance to environmental and medical interventions. Also, some patients may want to avoid medication side effects, costs, and long term use and are good candidates for immunotherapy. Immunotherapy may also prevent the development of asthma in children with allergic rhinitis.

Successful immunotherapy is associated with a shift from TH2 to TH1 CD4+ cells, immunologic tolerance, increases in allergen-specific IgG blocking antibodies, and variable levels of specific IgE. In order to have successful immunotherapy, the specific allergen must be elucidated and a standardized vaccine should be made. Weekly injections are continued with elevation of allergen dose until a maintenance dose is met. At this time, the injections will need to continue for at least a total of three to five years. With immunotherapy, comes the risk of anaphylaxis. To reduce this risk, an assessment of the patient's general medical condition is necessary, i.e. history of asthma. Physicians should be trained in and prepared for treating anaphylaxis.

## Conclusion

Allergic rhinitis plays a major role in the lives of millions of Americans. As otolaryngologists, we will see many patients with this disease or diseases that mimic it. We commonly prescribe medications for allergic rhinitis without knowing the inciting antigen or antigens. Allergy testing can complete the picture for the physician and can play a role in the treatment strategies used to combat this disease. Many of us do not receive this training in residency, and allergy testing can be somewhat of a mystery. Allergy testing plays a key role in immunotherapy, which is a viable alternative to medical management. Skin prick testing remains the most used test because of its ease of use, and its rapid results. It remains a great screening tool for allergic disease. Intradermal testing is slightly more invasive and does not yield much more useful information. In contrast, intradermal dilutional testing and modified quantitative testing are useful when a screening test is positive because they yield more information for starting doses of immunotherapy. RAST is also an integral part of allergy testing when contraindications to skin/dermal testing exist. There are still questions to be answered. In the future, we may use a combination of *in vivo* and *in vitro* techniques in addition to acoustic rhinometry or nasal provocation. Many of the antigens are currently under investigation to decipher which method of testing is the most sensitive and specific. Knowledge of allergy testing and immunotherapy is essential for all Otolaryngologists.

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