

**TITLE: Medical Management of Allergic Rhinitis**  
**SOURCE: Grand Rounds Presentation, University of Texas Medical Branch, Dept. of Otolaryngology**  
**DATE: April 30, 2009**  
**RESIDENT PHYSICIAN: Camysha Wright., MD**  
**FACULTY PHYSICIAN: Jing Shen, MD**  
**SERIES EDITOR: Francis B. Quinn, Jr., MD**  
**ARCHIVIST: Melinda Stoner Quinn, MS(ICS)**

## **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.

## **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>H</sub>2) 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<sup>+</sup> 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.

## **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, inter-tester variability, and non-standardized 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 radio-labeled. 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).

## **Immunotherapy**

The technique of allergen immunotherapy, also referred to as specific immunotherapy and which is currently employed as the 'standard method,' was introduced by Leonard Noon in 1911. Noon administered increasing doses of pollen extract at 7 to 14 day intervals employing changes in conjunctival sensitivity to guide increasing doses. As a result of injections over a period of several months he induced up to a 100-fold reduction in conjunctival sensitivity to grass pollen extract. Noon's work was continued by John Freeman. Freeman followed 20 treated subjects through the grass pollen season of 1911. Half had begun injections prior to the pollen season while the other half began injections after already experiencing hay fever symptoms. In both groups most patients reported decreased symptoms during the grass pollen season, and increased tolerance to conjunctival challenge was demonstrated in the 18 who were retested. (Nelson, 2008)

There is general agreement that very low-dose immunotherapy is not effective and that high doses are more effective than moderate doses. However, quantifying these terms is frustrated by the lack of meaningful and widely recognized measurements of potency for many extracts.

Johnstone and Crump (1961) placed 200 children with perennial asthma consecutively referred to a pediatric allergy clinic into one of four immunotherapy treatment groups. Children received injections either of placebo or extracts of all the inhalable allergens to which they were positive on skin testing but at maximum concentrations for each allergen of 1:10,000,000 w/v, 1:5,000 w/v, or 1:250 w/v. The children were followed for 4 years during which time they and their parents were unaware of the treatment group to which they had been randomized. The final evaluation was made by a clerk who was also unaware of each child's treatment group. Wheezing with exertion was reported by 54% in the placebo and very low-dose treatment groups, 31% receiving 1:5,000 w/v maintenance, and only 9% of those receiving 1/250 w/v maintenance treatment. Similar differences were reported for wheezing with respiratory infections.

A study by Franklin and Lowell confirmed the differences reported between high and intermediate doses of allergen reported by Johnstone and Crump. Twenty-five ragweed-sensitive subjects were paired by severity of symptoms. One in each group continued to receive the highest tolerated dose of ragweed extract (median dose 0.3 mL of a 1:50 w/v concentration) while the other received a concentration 20-fold less (median 0.3 mL of 1:1000 w/v). During the ensuing ragweed pollen season the group receiving the higher dose reported significantly fewer symptoms.

There are no absolute indications for specific immunotherapy with inhalant allergens. Even among allergy societies the recommended indications vary widely. The Expert Panel of the National Asthma Education and Prevention Program state in their Step Therapy of Asthma that subcutaneous allergen immunotherapy should be considered for patients with allergic asthma at Steps 2 through 4. US Dept of Health and NIH also endorsed the use of immunotherapy while listing certain considerations that should be weighed in making the decision. These considerations were: (1) the severity of the allergy to be treated, (2) the efficacy of the available immunotherapy, and (3) the cost, risk, and duration of pharmacologic therapy versus specific immunotherapy. Among the specific factors to consider were the greater efficacy in children and young adults, the greater likelihood of success in patients with a single sensitivity, and the risk in asthmatic patients unless they are asymptomatic with an FEV<sub>1</sub> of at least 70% of predicted.

Although authorities differ in whom they consider a suitable candidate for allergen immunotherapy, there are certain minimum requirements that should be agreed to by everyone. First are the diseases that are appropriately treated with allergen immunotherapy. In addition to *Hymenoptera* sensitivity, which is not further considered here, the primary diseases for which there is sound evidence that allergen immunotherapy is effective are allergic rhinitis and allergic bronchial asthma. The efficacy of specific immunotherapy in treating atopic dermatitis is not established, nor has it been successful in treating sensitivity to food.

In patients with allergic rhinitis or asthma, there are then three conditions which must be met: (1) the patient must have significant exposure to an allergen, (2) the patient must have demonstrated a significant level of sensitivity to the allergen, and (3) the pattern of symptoms must conform to the pattern of exposure. Significant exposure may be difficult to define; however, there are some general rules. Significant pollen exposures for a region can be determined by quantitative pollen sampling data. Significant levels of indoor allergens are being defined and, where there is a question of the presence of significant levels of an indoor allergen, allergen analysis of house dust specimens can be obtained for fees that are much less than the investment in allergen immunotherapy. Most of the recent studies which have demonstrated efficacy for immunotherapy have selected patients on the basis of positive prick skin tests and have often required positive in vitro tests for sensitivity as well. Finally, the pattern of allergen exposure should be capable of explaining the patient's pattern of symptoms. Treatment with one or two pollen extracts is unlikely to benefit patients with perennial symptoms without seasonal variation.

If it is accepted that each allergen group should be present in the treatment extract in roughly similar amounts, then botanical cross-reactivity must be considered either in selecting the extracts to be included in the skin test panel, in formulating the treatment extract, or both. (Nelson, 2008) The general patterns of botanical cross-allergenicity are that there is rarely significant cross-allergenicity between families, there is generally a degree of cross-allergenicity between tribes or genera of families, and there is generally a high degree of cross-allergenicity between species of the same genus. Clinically significant cross-allergenicity among the members of the same family is exemplified in the trees by the strong cross-allergenicity among members of the conifer family (cedar, cypress, juniper, arbor vitae)

## Writing an allergen extract (vaccine) prescription

Considerations in writing an allergy extract (vaccine) prescription are: (1) decision as to which allergen extracts to include, (2) maintenance doses which have been proven to be clinically effective, (3) potency of the allergen extracts available, (4) patterns of cross-reactivity, and (5) deleterious effects of some allergen extracts on others with which they may be mixed.

Extracts of some allergens have been shown to contain proteases that are capable of degrading the proteins in other extracts with which they may be mixed. Proteases have been reported in fungal and whole body insect extracts. Many fungal extracts and as well as cockroach extracts have been shown in mixture to cause loss of allergenic potency of a number of pollen extracts.<sup>1</sup> House dust mite extract did not appear to cause degradation of these pollen extracts, consistent with the low protease content of US mite extracts, which are made from mite bodies. Perhaps the best general rule is to not mix cockroach or fungal extracts with pollen, mite, or dander extracts.

Effective doses of the standardized extracts have been defined in terms of their major allergen content. The method of standardization which is employed in the United States (bioequivalent allergen units and major allergen content in FDA units) does not allow use of this information on dosing. However, representative lots of standardized extracts have been assessed for their major allergen content. Although this information allows an approximation of proven doses, the range of major allergen content for extracts labeled with the same US standardized potency is quite broad. However, in many cases the allergen extract manufacturer is able to provide major allergen potency for a particular lot of their extract. The unstandardized extracts can only be dosed based on analogy to standardized extracts or by what is known or suspected to be their potency. Thus unstandardized pollen extracts are assumed to approximate standardized grasses and ragweed in potency, while cockroach extracts have been shown to be quite weak. Clinical experience indicates that the 1:1000 v/v dilution of the maintenance vial is generally a safe starting concentration. (Moyer, 1985). Patients may also be prick skin tested with each dilution of extract mix and immunotherapy commenced with the most dilute concentration that yields a positive prick skin test.

Given the complexity of the decision-making process as to whether allergen immunotherapy is indicated, and the background knowledge that is required to formulate a proper allergen extract (vaccine), it is clear that this should be undertaken only by a physician with special training in the field.

It is critical that the vials containing the allergen extract (vaccine) for treatment be clearly and completely labeled. To ensure that the patient receives injections from the correct treatment set, the label should contain the patient's name and some other identifying information such as registration number or birth date. To ensure that the correct vial from the treatment set is used, the vial number, dilution, and a consistent color-coding of the caps are recommended – red (1:1 most concentrated), yellow (1:10 dilution of most concentrated), blue (1:100 dilution), green (1:1000 dilution) and silver (1:10,000 dilution). Additionally, the label should list the specific allergens contained in

the mixture and the expiration date of the contents. The latter two are particularly important when the vials are administered in another physician's office.

Once an allergen extract (vaccine) has been prepared for administration, its components are subject to loss of potency, particularly when in more dilute concentrations. This loss of potency can be retarded by the addition of preservatives such as 0.03% human serum albumin or glycerin in concentrations of 10% to 50%. Fifty percent glycerin is by far the best preservative, but its use is limited by the pain that accompanies its injection. The loss of potency is greatly accelerated by higher temperatures. Therefore, allergen extracts should be kept at refrigerator temperature at all times except when actually in use.

## **INJECTION SCHEDULES**

The initial build-up to maintenance is conventionally achieved by twice-weekly to biweekly injections of allergen extract. However, alternative schedules such as cluster daily, and rush have been employed. (Nelson, 2008) Once patients reach maintenance doses of their immunotherapy extract it is customary to give the maintenance injections at less frequent intervals, typically increasing over a period of time to once-monthly injections.

## **DURATION OF IMMUNOTHERAPY**

There are only a few studies that have adequately addressed the question of the duration of inhalant allergen immunotherapy. Benefit can be demonstrated after only a single series of pre-seasonal injections. There is a general perception, however, which is supported by prospective observations, that the clinical benefits may increase with continuation of the same dose over several seasons. Furthermore, it is felt that the benefit from a brief course of immunotherapy may be rapidly lost, while that from a longer course may persist after injections are discontinued. On this basis, general guidelines are that the course of allergen immunotherapy, if successful, should be continued until the patient has been symptom-free or has substantially reduced symptoms for 1–2 years and in most cases for a total of 3–5 years.

Three studies have examined the persistence of clinical improvement after discontinuation of immunotherapy with grass pollen extract. Specific immunotherapy to grass was discontinued after 3–4 years of treatment in 108 patients who had responded well to treatment. (Ebner, 1994) There was no control group. There was a progressive increase in the number of patients reporting a return of grass pollen symptoms which reached 31% by the third year but with no appreciable increase in the fourth and fifth years. Durham et al (1999) conducted a double-blind, placebo-controlled trial supported the findings in this open study. Thirty-two patients who had received immunotherapy with grass pollen extract for 3–4 years were randomized to either continue to receive grass extract or to receive placebo injections for the following 3 years. They were compared with untreated patients with grass-induced allergic rhinitis. Both those continuing to receive active immunotherapy and the placebo group had significantly fewer symptoms and need for medication than the untreated controls, and scores in the

active and placebo groups were virtually identical. Furthermore, both treated groups had similar persisting suppression of conjunctival sensitivity as well as immediate and late cutaneous reactions to grass pollen extract. Eng et al (2001) conducted an open study that followed a group of children treated with grass pollen immunotherapy and a control group out to 6 years after treatment was discontinued. Those who had received active treatment still had significantly smaller immediate skin tests, fewer new positive skin tests, less severe hay fever symptoms, and less asthma than the control group.

A recognized complication of allergen immunotherapy is the occurrence of localized and systemic reactions. The interval between allergen extract injection and development of a systemic reaction is of considerable importance, because it dictates the period of time that the patient should remain in the physician's office after receiving treatment. The more severe reactions tend to occur earlier; one study reported all severe reactions occurring within 30 minutes. (Bousquet, 1990) Information was available on the time of onset of 27 fatal reactions to allergen immunotherapy. (Bernstein, 2004) Twenty-three had their onset before 20 minutes, but four were reported to have begun more than 30 minutes following injection. After reviewing the fatalities associated with allergen immunotherapy, a committee of the American Academy of Allergy, Asthma and Immunology recommended 20 minutes as the customary waiting time after injections, but extension to 30 minutes for high-risk patients, primarily those with severe asthma (Reid, 1993).

The occurrence of a local reaction to immunotherapy was not found to be predictive of the occurrence of a systemic reaction. In a prospective study, local reactions were found to be an insensitive predictor of systemic reactions at the next allergen injection. (Bousquet, 1990) It was concluded that local reactions do not require dose adjustments.

## **Conclusions**

Immunotherapy is a viable option for pts not benefiting from medical management that works by altering one's immunologic response. Adjuvant therapies may be useful to maximize effect of immunotherapy.

## Bibliography

Asero R., Weber B., Mistrello G., et al: Giant ragweed specific immunotherapy is not effective in a proportion of patient sensitized to short ragweed: analysis of the allergenic differences between short and giant ragweed. *J Allergy Clin Immunol* 2005; 116:1036-1041.

Bernstein D.I., Wanner M., Borish L., et al: Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990–2001. *J Allergy Clin Immunol* 2004; 113:1129-1136.

Bousquet J., Hejjaoui A., Michel F.-B.: Specific immunotherapy in asthma. *J Allergy Clin Immunol* 1990; 86:292-305.

Bousquet J., Becker W.M., Hejjaoui A., et al: Differences in clinical and immunologic reactivity of patients allergic to grass pollens and to multiple-pollen species. II. Efficacy of a double-blind, placebo-controlled, specific immunotherapy with standardized extracts. *J Allergy Clin Immunol* 1991; 88:43-53.

Bousquet J., Hejjaoui A., Dhivert H., et al: Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. III. Systemic reactions during the rush protocol in patients suffering from asthma. *J Allergy Clin Immunol* 1989; 83:797-802.

Cox L., Li J.T., Nelson H., et al: Allergen immunotherapy: a practice parameter second update. *J Allergy Clin Immunol* 2007; 120:S25-S85.

Dolz I., Martinez-Cocerac C., Bartolone J.M., et al: A double-blind, placebo-controlled study of immunotherapy with grass pollen extract Alutard SQ during a 3-year period with initial rush immunotherapy. *Allergy* 1996; 51:489-500.

Davies J.M., Bright M.L., Rolland J.M., et al: Bahia grass pollen specific IgE is common in seasonal rhinitis patients but has limited cross-reactivity with ryegrass. *Allergy* 2005; 60:251-255.

Dykewicz, MS and S Fineman editors. “Diagnosis and management of rhinitis: Complete Guidelines of the Joint Task Force on Practice Parameters in Allergy, Asthma And Immunology.” *Annals Allergy, Asthma, and Immunology*(1998); Vol. 81, 478-518.

Ebner C., Kraft D., Ebner H.: Booster immunotherapy (BIT). *Allergy* 1994; 49:38-42.  
120.. Durham S.R., Walker S.M., Varga E.-M., et al: Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999; 341:468-475.

Eng P.A., Reinhold M., Gnehm H.P.E.: Long-term efficacy of preseasonal grass pollen immunotherapy in children. *Allergy* 2001; 57:306-312.

Esch R.E.: Manufacturing and standardizing fungal allergen products. *J Allergy Clin Immunol* 2004; 113:210-215.

Esch R.E.: *Role of proteases on the stability of allergic extracts.*

In: Klein R., ed. *Regulatory control and standardization of allergenic extracts*, Stuttgart: Gustav Fischer Verlag; 1990:171-177.

Freeman J.: 'Rush' inoculation. With special reference to hay-fever treatment. *Lancet* 1930; i:744-747.

Grier T.J., LeFevre D.M., Duncan E.A., et al: Stability of standardized grass, dust mite, cat and short ragweed allergens after mixing with mold or cockroach extracts. *Ann Allergy Asthma Immunol* 2007; 99:151-160.

Gungor, A et al. "A comparison of skin endpoint titration and skin prick testing in the diagnosis of allergic rhinitis." *ENT – Ear, Nose, and Throat Journal* (Jan 2004); Vol 83:1, 54-60.

Krouse JH and RL Mabry. "Skin testing for inhalant allergy: Current strategies." *Otolaryngology Head and Neck Surgery Supplement* (Oct 2003) Vol. 129:4

Li, JT and RF Lockey et al. "Allergen Immunotherapy: a practice parameter." *Annals Allergy, Asthma, and Immunology*; Volume 90, Jan 2003; 1-40.

Lowell F.C., Franklin W.: A double-blind study of the effectiveness and specificity of injection therapy in ragweed hay fever. *N Engl J Med* 1965; 273:675-679.

Johnstone D.E., Crump L.: Value of hyposensitization therapy for perennial bronchial asthma in children. *Pediatrics* 1961; 27:39-44.

Leavengood D.C., Renard R.L., Martin B.G., et al: Cross allergenicity among grasses determined by tissue threshold changes. *J Allergy Clin Immunol* 1985; 76:789-794.

Mabry, RL, BJ Ferguson and JH Krouse editors. Allergy: The Otolaryngologist's Approach. The American Academy of Otolaryngologic Allergy 2005.

Martin B.G., Mansfield L.E., Nelson H.S.: Cross-allergenicity among the grasses. *Ann Allergy* 1985; 54:99-104.

McKay SP, et al. "Intradermal positivity after negative skin prick for inhalants." *Otolaryngology Head and Neck Surgery* (2006); Vol. 135, 232-235

Meiser J.B., Nelson H.S.: Comparing conventional and acetone-precipitated dog allergen extract skin testing. *J Allergy Clin Immunol* 2001; 107:744-745.

Moyer D.B., Nelson H.S.: Use of modified radioallergosorbent testing in determining initial immunotherapy doses. *Otolaryngol Head Neck Surg* 1985; 93:335.

National Institutes of Health, National Heart, Lung, and Blood Institute. Global initiative for asthma: global strategy for asthma management and prevention. NHLBI/WHO workshop report, March 1993. Publication No 95-3659. January 1995.

Nelson H.S., Areson J., Reisman R.: A prospective assessment of the remote practice of allergy. Comparison of the diagnosis of allergic disease and the recommendations for allergen immunotherapy by board-certified allergists and a laboratory performing in vitro assays. *J Allergy Clin Immunol* 1993; 92:380-386.

Nelson H.S.: Effect of preservatives and conditions of storage on the potency of allergy extracts. *J Allergy Clin Immunol* 1981; 67:64-69.

135.. Nelson H.S., Ikle D., Buchmeier A.: Studies of allergen extract stability: the effects of dilution and mixing. *J Allergy Clin Immunol* 1996; 98:382-388.

Nelson H.S.: The use of standardized extracts in allergen immunotherapy. *J Allergy Clin Immunol* 2000; 106:41-45.

Nelson H.S., Lehr J., Rule R., et al: Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. *J Allergy Clin Immunol* 1997; 99:744-751.

Nelson, HS. Immunotherapy **for Inhalant Allergens. Chapter 95** .  
Adkinson: Middleton's Allergy: Principles and Practice, 7th ed. Mosby, 2008.

Nelson H.S., Oppenheimer J., Buchmeier A., et al: An assessment of the role of intradermal skin testing in the diagnosis of clinically relevant allergy to timothy grass. *J Allergy Clin Immunol* 1996; 97:1193-1201.

Peltier, JC and MW Ryan. "Comparison of intradermal dilution testing, skin prick testing, and modified quantitative testing for common allergies." *Otolaryngology Head and Neck Surgery* (2007); Vol.137,246-49

Platts-Mills T.A.E., Sporik R., Chapman M.D.: The role of indoor allergens in asthma. *Allergy* 1995; 50(Suppl 22):5-12.

Portnoy J., Bagstad K., Kanarek H., et al: Premedication reduces the incidence of systemic reactions during inhalant rush immunotherapy with mixtures of allergenic extracts. *Ann Allergy* 1994; 73:409-418.

Reid M.J., Lockey R.F., Turkeltaub P.C., et al: Survey of fatalities from skin testing and immunotherapy 1985-1989. *J Allergy Clin Immunol* 1993; 92:6-15.

Rouse, D, DL Park and T Sanford. "Allergy symptom response to intradermal testing based immunotherapy: A retrospective study of clinical practice." *Otolaryngology*

Head and Neck Surgery (2004); Vol 131, 220-24. Seehul et al. "Use of intradermal dilutional testing and skin prick testing: Clinical relevance and cost efficiency." *Laryngoscope* 116: Sept. 2006, 1530-1538

Shah, SB and IA Emmanuel. "Cost analysis of employing multi-test allergy screening to guide serial endpoint titration (SET) vs. SET alone." *Otolaryngology Head and Neck Surgery* (July 2003) Vol 129:1, 1-4.

Simons, JP et al. "Comparison of Multi-test II skin prick testing to intradermal dilutional testing." *Otolaryngology Head and Neck Surgery*; Vol. 130:5; 536-44.

Tipton W.R., Nelson H.S.: Experience with daily immunotherapy in 59 adult allergic patients. *J Allergy Clin Immunol* 1982; 69:194-199.

US Department of Health and Human Services. National Institutes of Health. National Heart, Lung, and Blood Institute. Expert Panel Report 3. Guidelines for the Diagnosis and Management of Asthma. 2007.

[www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm](http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm)

US Department of Health and Human Services. Public Health Service. National Institutes of Health. International consensus report on diagnosis and management of asthma. Publication No 92-3091. June 1992.

Uzzamann, A et al. "Acoustic rhinometry in the practice of allergy." *Annals Allergy, Asthma, and Immunology*; Vol. 97, Dec 2006; 745-752

Vailes L., Sridhara S., Cromwell O., et al: Quantitation of the major fungal allergens, Alt a 1 and Asp f 1, in commercial allergenic products. *J Allergy Clin Immunol* 2001; 107:641-646.

Van Metre Jr. T.E., Rosenberg G.L., Vaswani S.K., et al: Pain and dermal reaction caused by injected glycerin in immunotherapy solutions. *J Allergy Clin Immunol* 1996; 97:1033-1039.

Van Metre Jr. T.E., Adkinson Jr. N.F., Amodio F.J., et al: A comparison of immunotherapy schedules for injection treatment of ragweed pollen hay fever. *J Allergy Clin Immunol* 1982; 69:181-193.

Wood, RA et al. "A comparison of skin prick tests, intradermal skin tests and RASTs in the diagnosis of cat allergy." *Journal of allergy and clinical immunology* (May 1999) Vol 103:5 part 1, 773-779.

Wood R.A., Phipatanukul W., Hamilton R.G., et al: A comparison of skin prick tests, intradermal skin tests and RASTs in the diagnosis of cat allergy. *J Allergy Clin Immunol* 1999; 103:773-779.

Yoo T.-J., Spitz E., McGerity J.L.: Conifer pollen allergy: studies of immunogenicity and cross-antigenicity of conifer pollens in rabbit and man. *Ann Allergy* 1975; 34:87-93.