

CHAPTER 20

Allergen immunotherapy and standardization and stability of allergen extracts

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ALLERGEN IMMUNOTHERAPY

Clinical efficacy

Allergen immunotherapy (IT) enjoyed widespread use for 50 years before the first controlled studies showing efficacy in allergic rhinitis and allergic asthma were published. IT is allergen specific and results are dose dependent: high-dose therapy is superior to low-dose, and very-low-dose IT (as in Rinkel IT) is ineffective. Double-blind, placebo-controlled studies in allergic rhinitis have shown efficacy for crude short ragweed, antigen E, mountain cedar, *Cladosporium*, timothy, and orchard grasses. Studies in allergic asthma have shown benefits for northern grasses, cat and dog dander, house dust and dust mite, and *Cladosporium*. Local intranasal IT is effective with both ragweed aqueous and modified extracts. Oral IT with grass pollen capsules is not effective, nor is bacterial vaccine (respiratory stock vaccine). Several studies of IT have established a clinical benefit from placebo at about 30% of patients.

Immunologic changes

IT causes an initial rise in specific IgE levels in the serum and increased skin reactivity. Concomitant with the IgE rise is a rise in specific IgG levels. Further IgE rise is blunted, so that the usual seasonal peak of IgE is not attained while the IgG level continues to rise; skin reactivity diminishes concomitantly. The postseason rate of fall of the IgE level does not differ between patients receiving IT and those who are not, but because the seasonal rise is blunted, IT causes a more rapid decline of specific IgE levels over several years than seen in untreated patients.¹ In ragweed IT, the plateau IgG level correlates with the amount of antigen E given. Rush IT results in a quicker rise in IgG levels, although the plateau is the same as with conventional IT. Levels of IgG₁ and IgG₄ rise with

IT, IgG₁ peaking with the first 2 to 3 months; later on the IgG₄ response predominates. A large early increase in IgG₄ levels has been reported to be a poor prognostic sign for IT benefit.²

IT also results in a diminished leukocyte histamine release sensitivity with allergen challenge. IT diminishes the lymphoproliferative response seen with allergen stimulation in culture and stimulates development of specific suppressor T cells. IT blunts interleukin (IL)-2 receptors after allergen stimulation. A retrospective analysis of IT showed an inverse correlation of skin late-phase reactions with the IgG level.³

Safety

Adverse effects of allergen IT may be divided into those with and without IgE mediation. High-dose or "highest tolerated" IT is associated with frequent local reactions, occurring in approximately 40% of patients (2% to 4% of total injections). Systemic reactions occur in 7% to 17% of patients (0.2% to 0.3% of total injections). Local reactions are not good predictors of systemic reactions. Up to 40% of systemic reactions may be late in onset (≥ 35 minutes); however, in one study the severe delayed reactions all occurred within 60 minutes. Systemic reactions are more common in women than in men. IT in pregnancy is not associated with any greater risk to mother or child.⁴ Persistent asthma increases the likelihood of respiratory compromise and death due to an IT reaction.^{5, 6} Whether more systemic reactions occur during season is not clear.

β -Adrenergic antagonist therapy is associated with more severe and protracted anaphylactic reactions, but the incidence of adverse reactions does not appear to be greatly increased.⁷ Although IT may provoke high titers of specific IgG, no data indicate that this stimulation increases the likelihood of connective tissue or immune complex disease. A few case reports link allergen IT with acute serum sickness or the onset of polyarteritis nodosa. However, three studies of this

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therapy showed no increase of circulating immune complexes, hypocomplementemia, autoimmune disorders, or lymphoproliferative diseases over that seen in controls. Delayed reactions consistent with a type III, or Arthus, cause have been reported in some children receiving mold IT who had demonstrated precipitating antibodies to the molds before the start of IT.

STANDARDIZATION OF ALLERGEN EXTRACTS

Advantages and disadvantages

Standardization will allow for better quality control. Lot-to-lot variability from a single producer, as well as differences between commercial sources, would be minimized. This tighter range of potency and content should reduce the incidence of false-negative and false-positive results in diagnostic skin testing and result in smoother courses of IT with fewer factors contributing to potential adverse reactions. Comparisons of clinical research protocols using differing source materials will be facilitated.⁸

Disadvantages of standardized extracts are primarily the cost and effort of production. Because most crude extracts are complex mixtures of major and minor allergens and irrelevant material, truly complete characterization is almost unattainable.⁹ That standardized extracts will be more potent than conventional extracts and therefore will be more likely to produce reactions may in fact not be true. The practitioner using standardized extracts must assess how each extract compares with what he or she has previously been using.

Techniques of standardization

Allergen preparations may be characterized in terms of extracted materials: Noon units, weight/volume, and protein nitrogen units (PNUs). These terms are inadequate because not all extractable material is allergenically relevant; whereas most allergens are proteins, some significant allergens have a high carbohydrate component, which would be underestimated by PNUs. Isoelectric focusing and polyacrilamide gel electrophoresis may demonstrate potency, or degradation, by the presence or absence of protein bands. Immunologic evaluations for antigen or allergen content use either *in vitro* or *in vivo* techniques: quantitative immunoelectrophoresis including radioimmuno-electrophoresis, crossed immunoelectrophoresis, crossed radioimmuno-electrophoresis, RAST, enzyme-linked immunosorbent assay, skin test titration, and histamine equivalent prick test. The Food and Drug Administration (FDA) prefers extracts be standardized with titrated intradermal erythema response assessment in selected patients.¹⁰

Reference standards

The World Health Organization (WHO) has a series of international standard references against which other extracts may be compared. Presently, recognized international standards exist for *Dermatophagoides pteronyssinus* (house dust mite), *Phleum pratense* (timothy grass), *Ambrosia elatior* (short ragweed), *Betula verrucosa* (birch), and *Canis domesticus* (dog dander). Proposed standards are being pursued for *Alternaria tenuis*, *Cynodon dactylon* (Bermuda grass), *Lolium perenne* (perennial ryegrass), and *Felis domesticus* (cat dander).

STABILITY OF ALLERGEN EXTRACTS

The stability of allergen extracts affects both outcome of diagnostic skin testing and the administration of allergen IT. Determining extract potency through standardization techniques is meaningless if that potency cannot be maintained after manufacture. The loss of potency of an extract over time is compounded by storage temperature, dilution, relative volume of extract within the storage vial, type of allergen, diluent used, and preservatives used. Several mechanisms are involved, including chemical denaturation through oxidation; conformational changes due to pH, enzymatic degradation, or polymerization; and adsorption of allergens onto container surfaces. The effects of increased storage temperature may work through numerous mechanisms.^{11, 12} Allergen extracts are complex mixtures of allergens, some of which are heat labile and some of which are heat stable. Ragweed antigen E is heat labile, whereas some of the other ragweed allergens maintain potency despite boiling. Depending on a patient's sensitivity to different allergens, a denatured extract may have unpredictable results.

At present, lyophilization is the best technique available for maintaining extract potency. Once reconstituted, extracts without stabilizers rapidly lose potency. This loss is less with concentrated extracts (1:10 or 1:100 wt/vol) and more pronounced with dilute extracts ($\leq 1:1000$ wt/vol). Phenol has a deleterious effect and speeds degradation. Adsorption to the container is countered by using detergents such as Tween-80 or by increasing the protein content of the extract (stronger concentration, mixture of allergens, adding human serum albumin [HSA]). The use of 0.03% HSA as a reasonably effective stabilizer has not been associated with any evidence for the development of either anti-HSA immune complexes or anti-HSA IgE. Glycerol is the most effective stabilizer, especially in 50% solution; it is irritating, however, and cannot be used for either intradermal testing or IT. Solutions of 10% to 25% strength are less effective. The combination of 10% glycerol and 0.03% HSA has additive protection. Glycerol may act in

part as a competitive inhibiting substrate for sugar-cleaving enzymes; it has no activity against proteolytic enzymes. ϵ -Aminocaproic acid, as a potent enzyme inhibitor, has been shown to stabilize grass extracts, especially at higher storage temperatures. Tween-80, phosphate or bicarbonate buffers, and phenolated saline solution are relatively ineffective stabilizers. Even with glycerol and HSA, appreciable falloff occurs between 3 and 12 months; therefore, intradermal materials and IT extracts more dilute than 1:100 wt/vol should not be used beyond 3 months.

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Allergen immunotherapy and standardization and stability of allergen extracts

Allergen IT is associated with which of the following immunologic changes?

- a. Initial rise in specific IgE and rise in specific IgG levels
- b. Undiminished IgE seasonal peak with gradual fall of IgE level over several years

- c. Diminished production of IL-2 after allergen stimulation
- d. Initial fall in specific IgE and rise in specific IgG levels

Explanation: Both specific IgE and IgG levels rise in response to the start of IT; however, the typical seasonal rise of IgE is blunted. The natural history of untreated seasonal allergic rhinitis shows persistent seasonal peaks, with a gradual fall of IgE level over several years. Other immunologic changes include blunted IL-2 production with allergen challenge and the development of suppressor cells.

Answer: a and c.

Double-blind, placebo-controlled clinical trials of allergen IT have shown efficacy in which of the following circumstances?

- a. House dust asthma as assessed by diminished sensitivity to bronchial allergen challenge
- b. Mold-induced asthma or rhinoconjunctivitis after treatment with standardized *Cladosporium* extract as assessed by diminished bronchial or conjunctival allergen challenge and lower medication usage
- c. Cat dander asthma as assessed by delayed onset and diminished severity of symptoms with provocative cat exposure
- d. Ragweed allergic rhinitis with preseasonal local intranasal immunotherapy with short ragweed extract as assessed by symptom scores and medication usage

Explanation: Double-blind, placebo-controlled studies of allergic asthma have shown efficacy for grass, house dust, dust mite, *Cladosporium*, and cat and dog dander. Similar studies in allergic rhinitis have shown efficacy for ragweed, mountain cedar, grass, cat dander, and *Cladosporium*.

Answer: All are correct.

Increased severity or frequency of anaphylactic reactions to allergen IT has been reported in which of the following instances?

- a. Concomitant β -adrenergic antagonist therapy
- b. Women
- c. Asthma
- d. Pregnancy

Explanation: β -Blocker therapy potentiates acute anaphylaxis and makes its management more difficult; the reported increased incidence of anaphylactic reactions while taking β -blockers is based on weak ev-

idence. Asthma increases the likelihood of respiratory compromise with anaphylaxis. IT injection reactions are more common in women. IT in pregnancy is not associated with increased morbidity.

Answer: a, b, and c.

Which of the following statements concerning allergen extract stability is/are correct?

- HSA 0.03%, although an effective stabilizer, is a potent immunogen.
- Storing extracts at lower temperatures (4° C) maintains potency through suppression of enzymatic degradation.
- Phenol saline solution is a moderately effective stabilizer.
- Fifty percent glycerolated extracts are appropriate for epicutaneous skin testing, but not for intradermal testing.

Explanation: HSA in extracts has not been shown to result in detectable anti-HSA immune complexes. Phenol has a detrimental effect on stability, especially at weaker extract concentrations. Suppression of temperature-sensitive enzyme degradations is one of several mechanisms by which refrigeration may maintain extract potency. The irritant effect of glycerol is not apparent with epicutaneous testing.

Answer: b and d.

Which of the following statements concerning allergen extract standardization is/are true?

- An approved WHO international standard exists for dog dander but not for cat dander.
- The FDA recommended an in vivo standardization technique using titrated wheal responses in selected patients.
- Standardization will diminish the lot-to-lot and

producer-to-producer variability of allergen extracts.

- RAST evaluates allergenic activity of an extract, whereas IEF and PAGE assess antigenic content.

Explanation: Approval of the cat standard is pending. The FDA technique uses erythema, not wheal, response. Standardization should diminish variability. Isoelectric focusing and polyacrylamide gel electrophoresis evaluate protein content, not antigenic activity.

Answer: a and c.

Which of the following statements concerning allergen immunotherapy is correct?

- Efficacy of IT with the large majority of extracts that are commercially available is inferred from the results of a small number of adequate studies.
- Glutaraldehyde, formaldehyde, and alum-precipitated modified allergen extracts have been shown to be clinically effective.
- Grass-pollen asthma, but not ragweed-pollen asthma, has been shown to be responsive to IT.
- Although systemic adverse reactions to IT occur in less than 1% of all administered injections, any patient has approximately a 15% risk of having such a reaction.

Explanation: All of the statements are correct. Ragweed-alum-precipitated and pyridine-extracted preparations are not effective, but alum-precipitated only extracts are effective. Ragweed-pollen asthma has been difficult to document because of confounding factors of mold sensitivity.

Answer: All are correct.