

# Immunotherapy for Allergic Diseases

**Jay M. Portnoy**

*Section of Allergy, Asthma, and Immunology,  
Children's Mercy Hospitals and Clinics,  
Kansas City, MO 64108*

## Introduction

Allergen immunotherapy has been used for the treatment of allergic diseases such as rhinitis and asthma since the beginning of the 20th century (1). It generally is indicated for patients who have evidence of a clinically relevant IgE-mediated disease and in whom environmental control and pharmacotherapy inadequately treat the symptoms.

Immunotherapy is performed by injecting patients with mixtures of allergenic extracts. Such extracts consist of solutions of elutable substances derived from the raw materials that cause allergic reactions. Their manufacture, quality control, and regulation are discussed elsewhere in detail.

The most common allergen vaccines are those derived from pollens of trees, grasses, and weeds, as well as molds, animal danders, dust mites, and stinging insects of the order Hymenoptera (2). Unfortunately, vaccines from many potentially important exposures are not available. For example, there are no commercially available vaccines for many fungal ascospores or basidiospores (mushrooms), even though they frequently may be the dominant type of airborne bio-particulate during certain seasons.

A number of controlled trials have shown that immunotherapy reduces the severity of allergic symptoms and medication requirements in children as well as adults (3–5). Today, even with the availability of a variety of pharmaceuticals used to treat the symptoms of allergy, immunotherapy remains the only treatment that has the potential to affect the natural course of allergic diseases.

## Use of Standardized Vaccines

The need for standardized vaccines has been recognized for years. Units based on weight per volume (w/v) and the content of protein nitrogen units (PNUs) were initially used in an attempt to quantify potency. The problem is that biologic activity does not reliably correlate with the amount of elutable material or with the amount of protein nitrogen contained in an extract. For that reason, standardization using a biologic assay is necessary.

Today, standardized vaccines are used in therapeutic mixtures when they are available. Such extracts are labeled in BAUs (bioequivalent allergy units). Because dust-mite extracts were standardized before the establishment of the BAU designation, they are labeled in AUs (allergen units) adding another level of complexity to the already confusing plethora of potency designations. Approximately 10,000 AU/mL of dust mite is equal to 10,000 BAU/mL. Standardized vaccines are prepared as aqueous, glycerinated, and freeze-dried formulations and are compatible for mixing with nonstandardized products. Although some commonly used allergens such as grass pollens and cat are standardized, many others are not. Therefore mixtures of vaccines frequently contain constituents with different potency labels. It must be remembered that unstandardized products labeled in PNUs or weight per volume units show an inconsistent relationship with the biologic activity of the extract so it is not proper to relate BAUs with an equivalent weight per volume unitage.

There are two principal methods for standardizing allergen-vaccines: bioassays using quantitative skin tests, and in vitro assays using specific IgE with radio allergosorbent test (RAST) or enzyme-linked immunosorbent assay (ELISA) inhibition. Extracts in the United States are standardized by performing intracutaneous tests with three-fold dilutions of the extract to be standardized in a minimum of 15 highly allergic individuals. The arithmetic mean of the erythema reactions are measured. Extracts that produce an identical skin response at a 1:5 million dilution (w/v) are bioequivalent and are designated as 100,000 BAU (6). This method of standardization is different than the European method in which percutaneous (prick) testing is done using the wheal sizes and a histamine reference.

In vitro methods such as RAST or ELISA inhibition give an indication of total IgE-binding activity and are easier to perform than quantitative skin tests. Some extracts also are labeled in terms of micrograms of major allergen. Major allergens are antigens recognized by IgE contained in 50% of sera from selected clinically allergic subjects. Assays of specific allergens usually rely on "sandwich" assays using a mono-

**Table 1**  
**Guidelines for Maintaining Extract Potency**

- 
1. Store extracts at 4°C. Expose them to room temperature as infrequently as possible.
  2. Highly concentrated extracts are more stable than less concentrated ones.
  3. Lyophilized extracts are more stable than aqueous ones.
  4. Stabilizing diluents that preserve potency include 50% glycerine and 0.03% human serum albumin.
  5. An antibacterial agent such as phenol should be added to diluents.
- 

clonal antibody (MAb) to capture the major allergen and another MAb or human IgE directed to a different binding site or epitope to detect the amount captured. These assays generally are more sensitive than ELISA inhibition and may detect concentrations of major allergens measured in picograms .

### ***Storage of Allergen Vaccines***

Because the efficacy and risk of immunotherapy depends on use of vaccines with predictable biologic activity, it is important that they be stored under conditions that preserve that activity (*see* Table 1). The potency is affected by a number of factors including the passage of time, temperature, concentration, volume of the storage vial, and the presence of stabilizers and preservatives (7,8).

To minimize the rate of potency loss, aqueous products should be stored at 4°C because storage at higher temperatures (i.e., room temperature) results in rapid deterioration. Potency of concentrated vaccines (1:10 or 1:100 [w/v]) when kept at this temperature is relatively constant and allows the extract to be used until the expiration date that is on the label. Less concentrated vaccines are more sensitive to the effects of temperature and may not maintain their potency until the listed expiration date.

Highly concentrated vaccines are more stable than more dilute ones. Storage in a lyophilized form can enhance this. Aqueous vaccines should be mixed with solutions containing stabilizing agents such as 50% glycerine or 0.03% human serum albumin (HSA) to preserve potency. The mechanism for this potency preservation is unknown, though it has been hypothesized that they prevent adsorption to the glass vials. Glycerine at a concentration of 50% may also inhibit the activity of proteolytic and glycosidic enzymes that are present in certain vaccines. An antibacterial agent such as phenol should be also added as a preservative.

## **Preparation of Allergen Mixtures**

### ***Allergen Selection***

Because the efficacy of immunotherapy is specific for the antigen used (9), it is critical that all relevant antigens be identified when an extract mixture is formulated for a particular patient. Omission of important allergens to which the patient is sensitive may reduce the effectiveness of immunotherapy. On the other hand, inclusion of all allergens to which the patient has IgE as determined either by skin or in vitro methods might dilute each component sufficiently so that an effective dose is not given. Because it may not be practical to include allergens derived from all identified exposures in a particular mixture, a decision has to be made regarding the relative importance of various allergens as well as their immunologic cross-reactivities. This is done using a knowledge of the aerobiology of the region in which the patient lives, patterns of allergen exposure, and a detailed medical history focusing on identification of a relationship between the patient's symptoms and exposure to suspected allergens (10).

There currently are three methods for choosing allergens to include in a patient's mixture in common practice today. These include the all-inclusive method, the selective method, and the empiric method. The selective method involves inclusion of allergens that are associated with symptoms by history. Such allergens are most likely to be clinically relevant. Unfortunately, the history may be unreliable. It is common for patients who deny spring symptoms when questioned in the fall to experience them when seen the following spring. For this method to be used effectively, it may be necessary to observe a patient's response to medical treatment for a full year to determine the true seasonal pattern of symptoms before initiating immunotherapy.

Knowledge of the details concerning regional and local aerobiology is important when determining clinical relevance. These are available either in the news media, on various web sites, or through the Pollen and Mold network of the American Academy of Allergy, Asthma, and Immunology. Historical information about the region may be sufficient when pollen and mold seasons are consistent from year to year, though significant variations may occur depending on weather conditions. Though the relationship between day-to-day outdoor pollen and mold exposure and development of clinical symptoms is not always clear, symptoms that occur during periods of elevated exposure to allergens, in association with positive skin or in vitro allergy tests, provide good evidence that such exposures are relevant.

Determination of the significance of indoor allergen exposures is more difficult. Historical factors such the presence of a furry animal in the house, a history of water damage and mold, or a history of insect

infestation may be helpful for identifying exposures, however, such information is subjective and is often of uncertain reliability. As a result, it is often necessary to rely on allergen sensitivities alone to identify relevant allergens. It is also possible to send a sample of house dust from the patient's home to a reference laboratory for allergen measurement, however, this is expensive, is usually not covered by health plans, and the results are of uncertain usefulness.

The all-inclusive method involves addition of every antigen to which the patient has specific IgE. In practice, skin tests or RASTs are done and each of the antigens giving a "positive" reaction is added to the mixture. The disadvantage of this approach is that it may result in the addition of clinically irrelevant antigens. This is because it is hard to determine which allergens are truly irrelevant. This method is widely used because it is difficult to confirm the reliability of a clinical history. Histories generally are not detailed enough to discriminate between allergens when more than one is present such as when multiple exposures are present during a particular time of year. A patient who complains of fall symptoms and demonstrates IgE to two antigens that are present concurrently such as Ragweed and Kochia, for example, may be clinically sensitive only to one of the species. It would be difficult to determine which is clinically relevant without individual challenges. It is important to add only one representative species for immunologically cross-reactive antigens to prevent unnecessary dilution of each component.

Immunologic (and also allergenic) cross-reactivity is the tendency of different allergenic constituents to be recognized as the same or similar by the patient's immune system. Because one allergen can elicit the same immunologic responses as another cross-reacting allergen, it is not necessary or even desirable to include both in the same mixture. Doing so may result in addition of too much of the cross-reacting allergen leading to a possible adverse reaction, and unnecessary dilution of other allergens with resulting reduction in efficacy. Because treatment with vaccines derived from representative species has been shown to be efficacious, knowledge of immunologic cross-reactivities is essential when formulating vaccines. In general, the patterns of allergenic cross-reactivities among pollen and possibly fungal species follow their taxonomic relationships (10).

Empiric therapy involves the addition of antigens that are strongly suspected of causing symptoms based on the patient's history but for which there is no confirmatory evidence of specific IgE. The rationale is that the patient "must be allergic to this" so the treatment is given empirically. Because there is no evidence supporting this approach in the medical literature and there is a theoretical risk of sensitizing a

**Table 2**  
**Doing the Math<sup>a</sup>**

1. Express weight per volume as a fraction:

$$1 : N = \frac{1}{N}$$

2. Final concentrations in mixtures containing equal amounts of N components have a final concentration of

$$\frac{1}{N}$$

3. If Xcc of an antigen is added to a mixture with a final volume of Ycc then the final concentration of the antigen is:

$$(\text{initial concentration}) \times \frac{X}{Y}$$

4. Final concentrations of mixtures of mixtures are determined by multiplying the individual dilutions together. For example: if 0.5cc of a mixture of 5 extracts each with a concentration of 1:10 is added to a final volume of 5.0cc, the final concentration of each constituent would be.

$$\frac{1}{10} \times \frac{1}{5} \times \frac{1}{10} = \frac{1}{500} = 1 : 500$$

If the initial concentration were 10,000 BAU, the final concentration is

$$10,000 \text{ BAU} \times \frac{1}{5} \times \frac{1}{10} = 200 \text{ BAU}$$

---

<sup>a</sup>Because an optimal dose depends on the concentration of each constituent in the final mixture, it is important to do the math correctly. This involves a few simple rules as shown in the table.

patient to antigens to which they were not previously allergic, this method is not recommended.

### ***Dilution Effect***

An important consideration when formulating vaccines is the need to deliver an optimal dose of each of the allergenic constituents. Failure to do so may reduce the efficacy of immunotherapy. As one mixes more allergens together, there will be less of each in the final mixture due to the dilution effect. For this reason, it is usually necessary to restrict the number of different allergens in a single mixture while carefully calculating the final dilution of each constituent. The math consists of simple ratios as shown in Table 2.

### ***Interactions Between Vaccines***

Many allergen vaccines contain mixtures of proteins and glycoproteins. Some vaccines also contain proteolytic and glycosidic enzymes and there have been reports of interactions between vaccines

when mixed together (11,12). For example, extracts from fungi such as *Aspergillus* have been shown to reduce the IgE-binding activity of timothy-grass extract when mixed, though not of ragweed. There is no evidence that this decreased IgE-binding reduces their efficacy at it relates to immunotherapy because other mechanisms may be involved. Because these interactions have not been fully worked out, it may be desirable to keep vaccines that tend to have high proteolytic-enzyme activities such as those derived from fungi and insects separate from those with low activities such as pollens.

### ***Labeling of the Maintenance Vaccine***

#### *Identification Information*

A certain amount of information should be on each bottle label to avoid errors in administration. This should include:

1. The patient's name and birthdate.
2. The vaccine content in general form such as trees, grasses, weeds, and so on. It is not necessary (or practical) to list each constituent. Each distinct mixture should receive a letter designation in addition to a list of contents. A mixture can then be referred to as "bottle A" or "bottle B" in addition to "tree, grass, weed" or "dust, mold, mite."
3. The vaccine dilution and how that dilution is measured (i.e., [w/v], PNU's, BAU's). When more than one dilution is present such as during the build-up phase, bottle caps should be color-coded.
4. The bottle serial number: This should be unique to each mixture.
5. During the buildup phase, it may be helpful for tabs to be placed over each bottle top. These can be removed as each dilution is entered. By making it necessary to remove the tab prior to starting the next concentration, only one bottle at a time of each mixture is available for use. This may reduce the likelihood of administering an incorrect concentration.
6. Expiration date: Stock vials are labeled with an expiration date. Once these are mixed together, it is not known how long the mixture is stable. For this reason, there is no scientific basis for the determination of an expiration date of a new mixture. It is common practice to provide a 6–12-mo expiration for the most concentrated solutions because that is generally the time that elapses between clinic visits for patients on maintenance immunotherapy. A volume of vaccine that will last for the 6-mo period is therefore provided with each refill.

More dilute solutions should have shorter expiration times. Such vaccines are generally only used during the buildup phase of immuno-

therapy. For this reason, the expiration time of dilute vaccines should not be so short that a patient cannot finish using them before they expire. Because there is little solid data to base the time on, some flexibility can be permitted in the use of outdated vials. For example, it would be reasonable for a patient to receive their last injection from a given vial for 1–2 wk after the expiration date.

### ***Labeling of the Maintenance Vaccine Potency***

Once a patient's vaccine is prepared, it is important for a consistent labeling system to be used because errors in administration of injections are a common cause of systemic reactions. Unfortunately, there are several systems for designating the concentration of a maintenance bottle in common use today, making careful consistent labeling essential.

One system for labeling the maintenance vaccine potency is to list the final weight per volume concentrations of each constituent in the full strength extract. If they all are equal, then the vial may be simply labeled with the true weight per volume concentration (e.g., 1:100 [w/v]). If the final concentrations of the individual constituents are not the same (e.g., some are 1:100 [w/v] and some are 1:50 [w/v] or if the constituents are labeled in different units such as weight per volume and BAUs, then it doesn't make sense to label the final mixture with a single designated potency such as 1:100 (w/v). An alternative nomenclature is required in that situation. Because this situation will inevitably occur given the variation in optimal doses for different extracts and the increasing availability of standardized vaccines labeled in BAUs, it is best to avoid use of an arbitrary weight per volume designation because it can lead to confusion and potentially fatal errors.

Another method for labeling the maintenance concentration that is more universally applicable is to call it "full strength," "maintenance strength," or simply "concentrate" regardless of what the concentrations of its constituents are. The author's preference is to call it "maintenance concentrate" because a patient might not be able to tolerate the full strength or maintenance strength bottle and the word concentrate carries no implication regarding the need for its use. It is important to differentiate a patient's maintenance concentrate vial from the stock concentrate vials that are supplied by the allergen-product manufacturers. The latter consists of a single extract that is used for mixing to produce an individual patient's concentrate.

Because both systems of designating the maintenance concentration are in common use, it is important to clearly indicate dilutions from the full-strength vaccine. For example, a 10-fold dilution of a 1:100 (w/v) vaccine could be designated as 1:1000 (w/v) because that is the actual weight per volume dilution of the vaccine. A 10-fold dilution of a "maintenance concentrate" vial would be designated as 1:10 (v/v).

**Table 3**  
**Suggested Systems for Indicating Dilutions from the Full-Strength Extract**

| Dilution from patient's maintenance concentrate | (v/v) Label | Number | Color  |
|-------------------------------------------------|-------------|--------|--------|
| None                                            | 1:1         | 1      | Red    |
| 10-fold                                         | 1:10        | 2      | Yellow |
| 100-fold                                        | 1:100       | 3      | Blue   |
| 1000-fold                                       | 1:1000      | 4      | Green  |
| 10,000-fold                                     | 1:10,000    | 5      | Silver |

Whichever system of labeling one uses, it is important to keep a separate record of the vaccine's contents, including final dilutions of each constituent.

***Labeling of Dilutions from the Maintenance Vaccine***

During the buildup phase of immunotherapy, several dilutions of the patient's maintenance concentrate are present. For that reason, a redundant system for indicating those dilutions may help to avoid errors (*see* Table 3). These systems include the labeled dilution from the maintenance concentrate (in volume/volume), a numbering system, and a color-coding system. If a numbering system is used, the highest concentration should be numbered 1 rather than 4 or 5 because if larger numbers are used to indicate more concentrated vaccines, they would vary depending on how many dilutions are present. If a color-coding system is used, it should be modeled after the traffic light (green, yellow, red) colors with the highest concentration being red (stop), the next yellow (caution), and the most typical starting dilution being green (go). Because many primary-care offices receive bottles from different allergists, it would be helpful for a national system to be developed. It is best to reserve the use of letters to designate different series of extracts (e.g., pollens could be series "A," molds series "B," and so on).

**Administration of Immunotherapy**

***Build-Up Phase***

*Starting Dose for Build-Up Phase*

There are two phases of immunotherapy administration: the initial build-up phase when the dose and concentration of vaccine are slowly increased, and the maintenance phase, when the patient receives an optimal immunizing dose over a period of time. Two factors need to be considered when choosing the starting concentration of vaccine for the build-up phase of immunotherapy. The starting dose should not be too dilute or an unnecessarily large number of injections will be needed,

**Table 4**  
**Typical Buildup Schedule for Weekly Immunotherapy**

| Dilution (v/v) | Volume |
|----------------|--------|
| 1:1000         | 0.05cc |
|                | 0.15cc |
|                | 0.30cc |
|                | 0.50cc |
| 1:100          | 0.05cc |
|                | 0.10cc |
|                | 0.20cc |
|                | 0.35cc |
| 1:10           | 0.50cc |
|                | 0.05cc |
|                | 0.10cc |
|                | 0.20cc |
| Concentrate    | 0.30cc |
|                | 0.40cc |
|                | 0.50cc |
|                | 0.05cc |
|                | 0.10cc |
|                | 0.15cc |
|                | 0.20cc |
|                | 0.25cc |
|                | 0.30cc |
|                | 0.35cc |
|                | 0.40cc |
|                | 0.45cc |
|                | 0.50cc |

resulting in a delay in achieving an effective dose. On the other hand, the starting dose should not be too concentrated or the patient will be at increased risk of having a systemic reaction.

When choosing the starting dose, some allergists/immunologists perform intradermal skin tests using dilutions of the full-strength vaccine. The dilution giving < 10-mm diameter wheal is then considered to be an appropriate dilution for the initial injection. Other allergists/immunologists simply start at a sufficiently dilute concentration of the maintenance or full-strength vaccine so that few individuals will have a systemic reaction, though the disadvantage of this approach was mentioned earlier. There is no consensus on which approach to use, so it is up to the physician to choose the most appropriate approach for each patient. A usual starting dose would be 1:1000 of the full-strength concentrate, though a 10-fold more dilute dose is frequently used for patients who are highly sensitive as indicated by history or skin-test

reaction. It is not clear that adjustments to the starting dose needs to be made depending on the contents of the exact.

### *Schedules for Buildup Phase*

#### WEEKLY BUILDUP

A number of schedules have been described for the build-up phase of immunotherapy. The most common schedule is for increasing doses of vaccine to be administered one to two times per week. This weekly schedule is what most of the extract package inserts recommend, as shown in Table 4. With this schedule, a typical patient can expect to reach a maintenance dose in 4–6 mo depending on the starting dilution and the occurrence of systemic reactions. It is acceptable for patients to receive injections more frequently if desired provided that there is adequate spacing between shots. The interval between injections is empiric but may be as short as 1–2 d if there is some urgency to achieve a maintenance dose such as when there is an approaching allergy season.

Vaccines used during this phase usually consist of 3 or 4 10-fold dilutions of the maintenance concentrate vial (*see* Table 4). The dose generally is increased by 0.05 to 0.15cc per dose depending on a number of factors including the patient's sensitivity to the extract, history of prior reactions, and the concentration being delivered with smaller increments being given at higher concentrations. The volumes given generally start at 0.05cc and increase to 0.5cc. The next injection, given from a vial with a 10-fold higher concentration of vaccine, is usually 0.05cc, which represents the same total dose. This repeat dose is given to reduce the potential risk associated with giving vaccine from a more concentrated vial.

#### RUSH SCHEDULES

Many rush schedules have been described in an attempt to accelerate the build-up phase. Some schedules involve a cluster of injections given during the course of a day one or more times per week. This can permit the patient to reach a maintenance dose in as brief a time as 2–4 wk. More rapid rush schedules also have been described. The most accelerated schedule described for inhalant allergens involved giving 8 injections over the course of 6 h (13). Rush immunotherapy for stinging-insect venom has been described with schedules as brief as 90 min (14).

The main advantage of rush schedules is that they permit patients to attain their optimal maintenance dose more rapidly. Though there are anecdotal reports of more rapid symptomatic improvement with rush schedules, there are no studies that have documented such improvement. The advantage of rush immunotherapy comes at a cost because accelerated schedules are associated with an increased inci-

dence of local and systemic reactions (15). The risk of such reactions can be significantly reduced with premedication, however (16). Because systemic reactions with rush schedules have been reported to occur up to 2 h after the final injection, individuals receiving such immunotherapy should remain under physician supervision for a longer wait period on the injection day than the usual 20 min recommended for patients receiving injections on weekly schedules

### ***Premedication to Reduce the Risk of Immunotherapy***

Premedication prior to weekly immunotherapy with drugs such as antihistamines prior to each injection in an attempt to reduce the risk of systemic reactions has not been systematically studied. Such treatment frequently is discouraged because of the concern that it might mask a minor reaction that would otherwise alert the physician to an impending systemic reaction. Because some patients may be on an antihistamine as part of their allergy treatment when they receive their injection, there seems to be little basis for this concern. Other attempts to reduce systemic reactions, such as the addition of epinephrine to an extract, are also without basis and may delay the onset of a systemic reaction beyond the waiting time when the patient is in the physician's office, thus increasing their risk.

Premedication prior to rush immunotherapy has been shown to reduce the risk of a systemic reaction from approx 70% to 20% in a controlled study (16). The regimen used included a 3-d course of prednisone, an H-1 histamine receptor antagonist, and an H-2 histamine receptor antagonist. Anecdotal reports of further reductions in systemic reaction rates with addition of a leukotriene receptor antagonist have not been confirmed by studies published in the medical literature.

### ***Maintenance Immunotherapy***

#### *Maintenance Dose*

The maintenance dose of immunotherapy for a particular patient must be individualized. The prescribing allergist must balance the opposing concerns of treatment effectiveness with risk of reaction to the allergen injection. Low doses are less likely to be effective, and very low doses are ineffective. Administration of higher maintenance doses of immunotherapy increases the likelihood of clinical effectiveness. However, the risk of systemic reactions increases as the maintenance dose of immunotherapy increases. Furthermore, highly sensitive patients may be at increased risk of systemic reactions to immunotherapy injections.

Clinical studies suggest that maintenance immunotherapy injections of 5–20 mcg of major allergen per injection are effective and rea-

sonably well tolerated in most patients. When standardized allergic extracts (vaccines) are used, the recommended maintenance dose of immunotherapy is 1000–2000 BAUs. Highly sensitive patients or those who develop significant systemic reactions to immunotherapy doses during the build-up period may require lower doses of immunotherapy injections. Patients who tolerate 1000–2000 BAU (or 5–20 mcg major allergen) maintenance injections but who continue to experience allergic symptoms may benefit from higher doses of immunotherapy. Though early improvement in symptoms has been documented with immunotherapy, its long-term benefit appears to be related to the cumulative dose of vaccine given over time.

Regardless of dose schedule, some patients will be unable to progress to the optimal or predetermined maintenance dose because of local reactions or systemic reactions to the allergen vaccine. These individuals should be maintained on the highest tolerated dose. The patient's maintenance dose is thereafter administered every 1–2 wk until clinical improvement is noted, and thereafter, the interval between injection visits can be increased to every 3–4 wk. An interval of 2–4 wk between injections is recommended for most patients with inhalant allergen immunotherapy, contingent upon the maintenance of clinical improvement. Some patients do well with injections every 6 wk; whereas in other patients greater efficacy and/or fewer reactions may occur if the interval between injections is only 1 wk. Therefore, it is essential that the interval between allergen-immunotherapy injections be individualized in order to provide the greatest efficacy and safety for each patient. If clinical improvement is not apparent after 1 yr of therapy, discontinuing immunotherapy should be strongly considered and other treatment options pursued. If allergen immunotherapy is effective, treatment is usually continued for 3–5 yr or longer, depending on the patient's ongoing response to treatment and other clinical and practical considerations.

## ***Techniques for Administration of Immunotherapy***

### *Documentation*

It is very important that adequate documentation of the immunotherapy be preformed. The following information should be entered into the patient's medical record:

1. The dilution from the maintenance concentrate vial (in v/v) or if the absolute concentration is used it should be indicated either in BAUs or w/v.
2. The letter of the vial and its contents should be used if there is more than one pre-mixed vial of vaccine. Each should receive a unique

letter and the contents of the vial should be generally denoted (e.g., trees, grasses, weeds, etc.). Because it often is not practical to state each constituent on the allergy-shot form or vial label, this should be documented in the medical record.

3. The patient's name should be clearly documented along with the patient's medical record number, and other appropriate identifying information such as birthdate, telephone number, address, and so on. If there are two or more patients with the same name, this should be somehow noted on the form. It may be desirable to include a picture of each patient to reduce the likelihood of an error.
4. The scheduled volume of vaccine and the amount that was actually given should be recorded in milliliters of extract. It is desirable to record the next dose to be given at the time of injection because this is when one is most aware of circumstances that might require dose adjustments. In the case of routine build-up schedules, the anticipated dose schedule may be indicated in advance.
5. It is useful to record which arm the injection is given in. This may help to determine which vaccine is responsible if there is a local reaction. Because local reactions do not reliably correlate with systemic reactions, the presence of such a reaction may not be useful if there is a systemic reaction.
6. In some offices, peak flow are performed before and occasionally 20–30 min after each injection, particularly for asthmatics or patients with a history of previous systemic reactions. If a patient's peak flow is 20% below their baseline, it may be desirable to evaluate their clinical condition prior to administration of the injection.
7. A history of any reaction that may have occurred following the previous injection should be recorded. Dose adjustments may be necessary if reactions are frequent or severe.
8. It is desirable for any reaction that occurs 20–30 min after the injection to be recorded. This may be used to determine whether a patient is having reactions (both local and systemic) so that the dose of immunotherapy can be adjusted if needed.
9. Patients who have fever or any significant systemic illness should generally not receive an injection on that day. Documentation of the patient's clinical condition indicates that they were asked about such illness. If a reaction does occur, one can be reasonably certain that it was not due in part to a pre-existing illness.

All procedures and treatments should be properly documented in the medical record along with any adjustments from the standard schedule and the reason for them.

### *Procedures for Giving Immunotherapy*

Because errors in administration of injections were reported in 6 of 17 instances in one study, careful attention to details such as reading all labels is important. If multiple dilutions are present, the concentration of the vaccines may be determined using the following cues: color of vial top, the labeled dilution, the color of the fluid (more concentrated vaccines tend to be darker), and the vial number if used. There may be confusion if some extracts are labeled in weight per volume while others are labeled in protein nitrogen units (PNUs) or BAUs. This is because w/v numbers increase while PNU and BAU numbers decrease with increased dilutions. It may help to place a seal over higher potency vaccines until they are used to reduce the risk of errors in administration.

It is customary to provide separate injections from each mixture of allergens if more than one is to be given. These are usually administered in different arms, which are alternated on a weekly basis. An alternative approach is to draw the different mixtures into a single syringe and give one injection. This may be preferable for young children who are reluctant to receive multiple injections.

The skin should be wiped with an alcohol swab. This does not sterilize the area but it does remove gross contamination from the skin surface. It is customary to use a 26–27-gauge syringe with a 1/2" non-removable needle for allergy injections. Syringes specifically designed for immunotherapy are available from medical supply companies. The use of a removable needle syringe may reduce the accuracy of dose measurements at low volumes owing to the dead space in the needle hub. There should not be air space in the hub of the syringe. Air can be expelled by flicking the syringe with a finger while holding the needle end up.

Immunotherapy is given subcutaneously. This type of injection results in formation of a reservoir of extract that is slowly absorbed over time. The goal is to avoid absorption that is too rapid leading to a systemic reaction. Intracutaneous injection will generally result in a large local reaction whereas intramuscular injection may lead to rapid absorption and/or local reactions

The injection should be given in the posterior of the middle third of the upper arm. This location tends to have a greater amount of subcutaneous tissue than adjacent areas. Enough room should be left to permit tourniquet placement above the injection site to slow systemic absorption if a systemic reaction occurs. The skin should be pinched to avoid intramuscular injection and to increase access to the subcutaneous tissues. The needle should then be inserted bevel up at a 90° angle to the skin surface.

Aspiration should be done before the injection to check for blood return. If blood is seen, the needle should be removed and the syringe discarded in an appropriate container ("sharps" box). Another dose of extract should be drawn into a new syringe and a new site chosen for the injection. Removal of the syringe when blood is seen reduces the likelihood of intravenous administration, which could lead to a systemic reaction. The syringe and needle with the blood in it must be discarded. If it were not then when the site is changed and reaspirated, one could not determine whether a blood vessel is again entered.

The plunger should be depressed at a rate that does not result in wheal formation or excessive pain. Mild pressure should be applied to the injection site for about 1 min immediately after removal of the needle. This reduces leakage of the extract, which could cause local reactions.

### ***Monitoring for Efficacy***

During a course of immunotherapy, it is desirable to determine whether it has been effective. This can be done by measuring of any one or a combination of objective and subjective parameters.

Objective measurements of efficacy include *in vitro* responses such as the demonstration of an increase in specific IgG or reduction in specific IgE or IgG4 (17,18). Though these changes have been seen in studies of immunotherapy, they have not been found to reliably predict clinical effectiveness. Basophile histamine release has also been shown to decrease during immunotherapy (19), however, this was not specific to the antigen being administered and it has not been shown to correlate with clinical effectiveness (20). Antigen-specific CD8-positive lymphocytes that are also CD25-positive (interleukin-2 [IL-2] receptor) have been shown to increase in children who had a good response to immunotherapy with house dust extract as compared to a control group (21). Reduction of the low-affinity IgE receptor (CD23) has also been demonstrated with immunotherapy, but these changes do not reliably correspond to clinical improvement (22). Release of tumor necrosis factor (TNF) and IL-1 in response to allergen exposure along with histamine release also decrease with specific immunotherapy and this does appear to correlate with effectiveness (23). In another study, CD4 lymphocytes that also expressed messenger RNA for interferon- $\gamma$  were increased in patients who were receiving immunotherapy (24). Release of IL-12 or reduction in IL-4 and 5 are also possible predictors of efficacy, though they are not practical for routine clinical use.

Objective physiologic measurements include determination of pulmonary function including spirometry or serial peak flows, bronchial challenges either with antigen or a nonspecific agent such as metha-

choline. Sensitivity to the allergen on titrated percutaneous testing (25,26), titrated conjunctival challenge (27), and titrated nasal challenge (26) following immunotherapy and preceding the pollen season have been shown to correlate with symptoms of rhinitis occurring during natural pollen exposure. Though it does correlate with clinical efficacy, reduction in the skin-test response to aeroallergens after a period of immunotherapy can be highly variable in clinical practice and does not necessarily indicate good clinical response. For that reason, repeated skin testing should not routinely be used to monitor the effectiveness of immunotherapy.

Though it is desirable to monitor the effectiveness of immunotherapy using objective measurements, many of the aforementioned are not practical for routine clinical use. For that reason, most allergists rely on subjective measurements. Such indicators of efficacy include a patient's report that they feel better during a season when they normally would be symptomatic or a report that they require fewer medications. Though these subjective measurements are the most common means by which physicians judge the results of immunotherapy, they are not very reliable given the strong placebo effect associated with the treatment. A more reliable means for determination of efficacy is to use clinical symptom scores and the amount of medication required to control symptoms and maintain peak flows or pulmonary function tests within acceptable limits because these have been validated in controlled clinical trials. Calculation of symptom or medication scores can be used to assess these more quantitatively. A meta-analysis by Abramson et al. (3) included studies that used many of these including symptom scores, medication scores, and bronchial hyperreactivity to demonstrate effectiveness.

To determine a course of immunotherapy has been effective by whatever means the clinician chooses, that there have not been an excessive number of adverse reactions to the injections, and to answer questions that may have arisen during the course of immunotherapy, it is desirable to monitor each patient at 6- to at most 12-mo intervals.

## Long-Term Outcomes

Though pollen immunotherapy is effective in selected patients with IgE-mediated seasonal allergic rhinitis, there is a great deal of uncertainty about whether there is long-term benefit after the discontinuation of treatment. Durham (28) conducted a controlled trial of the discontinuation of immunotherapy for grass-pollen allergy in patients in after 3–4 yr of successful treatment. After 3–4 yr off of immunotherapy, scores for seasonal symptoms and the use of rescue anti-allergic medication remained low following the discontinuation of

immunotherapy, and there was no significant difference between patients who continued immunotherapy and those who discontinued it. Although there was a tendency for immediate sensitivity to allergen to return late after discontinuation, there was a sustained reduction in the late skin response and associated CD3+ T-cell infiltration and IL-4 messenger RNA expression. The authors concluded that immunotherapy for grass-pollen allergy for 3–4 yr induces prolonged clinical remission accompanied by a persistent alteration in immunologic reactivity.

## Conclusions

Immunotherapy continues to be an important component in the treatment of allergic diseases. It remains the only mode of treatment that has exhibited the potential for modifying the natural history of allergic diseases and possibly for preventing them from occurring at all. Though many aspects of its administration still need to be studied in a scientifically rigorous manner, it has proven to be effective even when given with nonstandardized methods. As a result, it continues to be important for physicians to learn the art of giving effective immunotherapy in addition to the ever-expanding science.

## References

1. Noon, L. (1911), *Lancet i*, 1572–1573.
2. Bousquet, J., Hejjaoui, A., and Michel, F. (1990), *J. Allergy Clin. Immunol.* **86**(3 Pt 1), 292–305.
3. Abramson, M., Puy, R., and Weiner, J. (1995), *Am. J. Respir. Crit. Care Med.* **151**, 969–974.
4. Ross, R., Nelson, H., and Finegold, I. (2000), *Clin. Therapeut.* **22**(3), 329–341.
5. Ross, R., Nelson, H., and Finegold, I. (2000), *Clin. Therapeut.* **22**(3), 342–350.
6. Turkeltaub, P. (1999), Allergen vaccine unitage based on biological standardization: clinical significance, in *Allergens and Allergen Immunotherapy*, 2nd ed. Lockey, R. and Bukantz, S., eds. Marcel Dekker, Inc., New York. pp. 321–340.
7. Nelson, H. (1981), *J. Allergy Clin. Immunol.* **67**, 64–69.
8. Hjorth, N. (1958), *Acta Allergol* **12**, 316–335.
9. Lowell, F. and Franklin, W. (1965), *N. Engl. J. Med.* 675–679.
10. Weber, R. and Nelson, H. (1985), *Clin. Rev. Allergy* **3**, 291–318.
11. Esch, R. (1991), *Arb Paul Ehrlich Institut* **85**, 171–179.
12. Nelson, H., Ikle, D., and Buchmeier, A. (1996), *J. Allergy Clin. Immunol.* **98**, 382–388.
13. Sharkey, P. and Portnoy, J. (1996), *Ann. Allergy Asthma Immunol.* **76**(2), 175–180.
14. Bernstein, J., Kagen, S., Bernstein, D., and Bernstein, I. (1994), *Ann. Allergy* **73**, 423–428.
15. Portnoy, J., King, K., Kanarek, H., and Horner, S. (1992), *Ann. Allergy* **68**(6), 493–498.
16. Portnoy, J., King, K., and Kanarek, H. (1994), *Ann. Allergy* **73**(5), 409–418.
17. Gleich, G., Zimmermann, E., Henderson, L., et al. (1982), *J. Allergy Clin. Immunol.* **70**, 261–271.

18. Peng, Z., Naclerio, R., Norman, P., et al. (1992), *J. Allergy Clin. Immunol.* **89**, 519–529.
19. Sadan, N., Rhyne, M., Mellits, E., et al. (1969) *N. Engl. J. Med.* **280**, 623–627.
20. Lichtenstein, L. and Levy, D. (1972), *Int. Arch. Allergy Appl. Immunol.* **42**, 615–626.
21. Bonno, M., Fujisawa, T., Iguchi, K., et al. (1996), *J. Allergy Clin. Immunol.* **97**, 680–688.
22. Lkjaic-Turkalj, M., Cvoriscec, B., Tudoric, N., et al. (1996), *Int. Arch. Allergy Appl. Immunol.* **111**, 188–194.
23. Wang, J-Y, Lei, H-Y, and Hsieh, K-H. (1992), *J. Asthma* **29**, 193–201.
24. Varney, V., Hamid, Q., Gaga, M., et al. (1993), *J. Clin. Invest.* **92**, 644–651.
25. Ohman, J. J., Findlay, S., and Leitermann, K. (1984), *J. Allergy Clin. Immunol.* **74**(3 Pt 1), 230–209.
26. Bousquet, J., Maasch, H., Martinot, B., et al. (1988), *J. Allergy Clin. Immunol.* **82**, 439–446.
27. Lofkvist, T., Agrell, B., Dreborg, S., et al. (1994), *Allergy* **152**, 100–107.
28. Durham, S., Walker, S., Varga, E., Jacobson, M., O'Brien, F., Noble, W., et al. (1999), *N. Engl. J. Med.* **341**(7), 468–475.