Allergy Diagnosis

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The diagnosis of IgE-mediated allergy is based on the confirmation of a typical history of allergic symptoms by diagnostic tests. IgE is the major isotype of anaphylactic antibodies, and although, theoretically, IgG4 can also act as a reagin, its clinical importance is not significant. Thus in vivo and in vitro tests are used in the diagnosis to detect free or cell-bound IgE (Fig. 1). The diagnosis of allergy has been improved by allergen standardisation, which provides satisfactory extracts for both in vivo and in vitro tests for most inhalant allergens, and the introduction of recombinant allergens. In the present chapter, neither non-specific challenges nor food and drugs will be considered, since they are presented in detail in other chapters.

Skin Tests

Immediate-reading skin tests are widely used to demonstrate an IgE-mediated allergic reaction and represent a major diagnostic tool. If properly performed, they yield useful confirmatory evidence for a diagnosis of specific allergy. As they are complex to perform and interpret, it is recommended that they be carried out by experienced personnel [1].

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R. Pawankar et al. (eds.), *Allergy Frontiers: Diagnosis and Health Economics*,
DOI 10.1007/978-4-431-98349-1_2, © Springer 2009

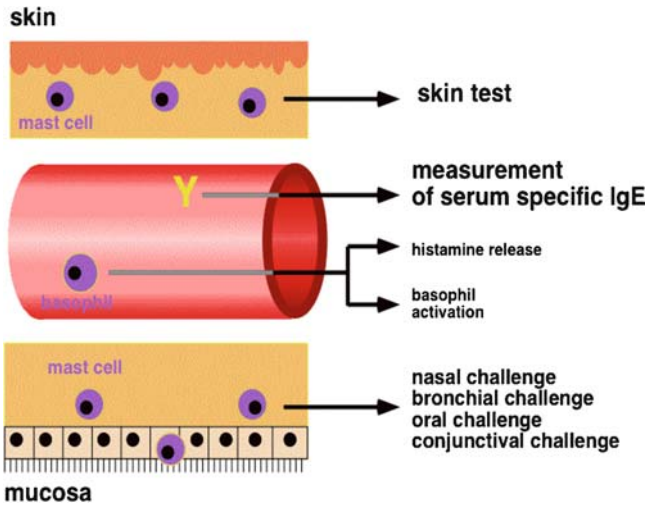

Fig. 1 Diagnosis of IgE-mediated allergy

Methods

Skin Testing Methods

Several methods of skin testing are available.

- **Prick and puncture tests** (SPT)

There is a high degree of correlation between symptoms and the results of both prick tests and provocative challenges. The modified prick test introduced by Pepys [2] is the current reference method, although the variability of this test has been shown to be greater than that of the intradermal test. Puncture tests with various devices [3–7] were introduced to decrease the variability of prick tests. Opinions concerning these so-called standardised methods vary according to the skill, experience and aims of the investigator. They are highly reproducible. Prick tests should be 2 cm apart.

- In some instances (e.g. weak allergen solution), *intradermal skin tests* may be employed. Although they are more sensitive than prick tests, they may induce some false-positive reactions and correlate less well with symptoms [8]. Intradermal tests are less safe than prick tests, since, although rarely, systemic reactions may occur [9]. Particular care should be taken in patients treated with β-blocking agents, which may increase the risk of systemic reactions. Intradermal tests are not considered useful for the diagnosis of inhalant allergy when standardised extracts are available [1, 9–11]. As a general rule, the starting dose of intracutaneous extract solutions in patients with a preceding negative prick test should

range between 100- and 1,000-fold dilutions of those used for prick-puncture tests [12]. The European Academy of Allergology and Clinical Immunology [13] and the US Joint Council of Allergy Asthma and Immunology [12, 14] therefore recommend skin prick-puncture tests for the diagnosis of IgE-mediated allergic diseases and for research purposes.

- **Prick–prick tests:** Prick plus prick tests with fresh foods, mainly fruits and vegetables, were introduced [15, 16] because the commercial extracts of some foods are not sufficiently sensitive or may not even be available.
- **Atopy patch tests:** The atopy patch test is a procedure involving epicutaneous patch tests with allergens known to elicit IgE-mediated reactions and the evaluation of eczematous skin lesions after 24–72 h in patients with aeroallergen- and food-triggered atopic dermatitis [17]. It has been standardised with regard to the use of vehicle and dose response relationships [18, 19]. Although there is increasing evidence that a small subset of patients with atopic dermatitis show atopy patch test positivity and serum-specific IgE negativity to the same allergen, as far as food allergy is concerned, the atopy patch test still requires standardisation [20, 21]. It may also be difficult to differentiate between irritative and allergic reactions [20, 21]. Wheat gluten in particular has been suspected of causing false-positive (irritant) reactions [22]. The diagnostic performance of this test with food allergens can be evaluated by comparing the results with double-blind, placebo-controlled food challenges (DBPCFC). There is a task force to study standardisation of the challenge procedures for delayed reactions [21]. Instead, the problem with aeroallergens is that a 'gold standard' provocation test in atopic eczema does not exist [23].

Negative and Positive Control Solutions

Because of inter-patient variability in cutaneous reactivity, it is necessary to include negative and positive controls in every skin test study. The negative control solutions are the diluents concerned. Rare dermographic patients will have wheal-and-erythema reactions to the negative control. The latter will also detect traumatic reactivity induced by the skin test device (with a wheal which may approach a diameter of 3 mm with some devices) and/or the technique of the tester [12]. Any reaction at the negative control test sites will hinder interpretation of the allergen sites [12].

Positive control solutions are used to detect possible suppression by medication or disease, exceptional patients who are poorly reactive to histamine or variations in technician performance.

The usual positive control for prick-puncture testing is histamine dihydrochloride, used at a concentration of 5.43 mmol/l (or 2.7 mg/ml, equivalent to 1 mg/ml of histamine base) [1]. Wheal diameters with this preparation range from 2 to 7 mm. However, a tenfold greater concentration is more appropriate [24], with a mean wheal size ranging between 5 and 8 mm. For the intradermal test, the concentration routinely used is 0.0543 mmol/l. The mean wheal size elicited ranges from 10 to 12 mm. Mast cell secretagogues such as codeine phosphate 2.5% [5] or 9% [25] may

also be used. Histamine-induced skin reactions reflect vascular reactivity and are useful to determine whether histamine antagonists are present, while codeine skin reactions are a function of both mast cell reactivity and vascular responsiveness. In this, the reaction pathway to codeine is comparable to that of allergens, which bind to specific IgE on high-affinity receptors on the mast cell surface and stimulate mediator release, including histamine. The use of both substances therefore may provide a more accurate impression of the reactivity of the individual than one substance alone [26].

Skin Tests with Recombinant Allergens

Current diagnosis of allergy relies on natural extracts, which may lack standardisation and/or be degraded rapidly in solution. Recombinant DNA technology allows production of pure biochemically characterised proteins. Skin tests with recombinant allergens have been available since the 1990s for pollens [27], moulds such as *Aspergillus* [28], mites [29], venoms [30], and latex [31]. Skin tests with recombinant and natural allergens have a similar value [32–34] if the recombinant allergens have been well selected and represent all or most epitopes of the natural allergen [35, 36].

Allergy diagnosis based on allergenic molecules is important in patients with multiple pollen sensitisation [37], since this condition appears to be determined by sensitisation to defined allergenic components (panallergens) or by pollen of multiple species [38]. Detection of IgE to non-panallergenic molecules using skin tests and/or serum IgE allows more important allergenic sources to be identified.

Food allergens are usually non-standardised and unstable in solution. Recombinant allergens should be useful for the diagnosis of food allergy such as apple [39], celery [40], peanut [41], and cherry [42]. Skin tests with recombinant food allergens can be an alternative to prick–prick tests with foods.

Grading of Skin Tests and Criteria of Positivity

Skin tests should be read at the peak of their reaction by measuring (in mm) the wheal and the flare approximately 15 min after the performance of the tests. Late-phase reactions are not recorded because their exact significance is not known [12, 13]. Some scoring systems have been proposed and may be used in daily practice.

In the USA, for example, for skin-prick tests: neg = 0 reaction, 1 + = 1 mm wheal above saline control; 2 + = 1–3 mm wheal above saline control; 3 + (the first point we consider a positive reaction) = 3–5 mm wheal above saline control plus an accompanying flare; 4 + = > 5 mm wheal above saline control, plus an accompanying flare.

For prick tests, when the control site is completely negative, small wheals of a mean diameter of at least 3 mm of the negative control represent a positive immunological response [1, 2], but these reactions do not necessarily imply the presence of a clinical allergy [1].

Factors Affecting Skin Testing

Skin reaction is dependent on a number of variables that may alter the performance of skin tests:

- *The quality of the allergen extract* is important. When possible, allergens that are standardised by using biological methods and that are labelled in biological units should be used [12, 13]. Recombinant allergens can also be used effectively (see above).
- *Age* is known to affect the size of the reaction [1], but positive skin-prick tests can be found early in infancy [43, 44]. In old age, the size of skin tests decreases [45].
- *Seasonal variations* related to specific IgE antibody synthesis have been demonstrated in pollen allergy [46]. Skin sensitivity increases after the pollen season and then declines. This effect has some importance in patients with low sensitivity [47] and/or in patients sensitised to allergens such as cypress pollen [48].
- *Drugs* affect skin tests and it is always necessary to ask patients about the drugs they have taken. Some drugs such as astemizole (no longer available in many countries) can depress or abolish responses to skin tests for a period of up to 6 weeks (for review see [1]). Discontinuing other H1-antihistamines 1 week before is required [1]. Montelukast does not appear to reduce skin test reactivity [49] and does not need to be discontinued before skin testing. Butterbur *Petasites hybridus*, a herbal remedy for the treatment of allergic rhinitis, does not produce any significant effects on the histamine and allergen cutaneous response [49].
- Patients with dermographism (urticaria) or widespread skin lesions should not be tested by prick puncture tests. Intradermal tests with the proper negative control may sometimes be feasible.

Interpretation of Skin Tests

Carefully performed and correctly interpreted skin tests with high-quality allergen extracts and a battery that includes all relevant allergens of the patient's geographic area are a simple, painless and highly effective method. Therefore, skin testing represents one of the primary tools for allergy diagnosis.

Both false-positive and false-negative skin tests may occur because of improper technique or material. False-positive skin tests may result from dermographism or may be caused by 'irritant' reactions or a non-specific boost from a nearby strong reaction [1].

False-negative skin tests can be caused by: extracts of poor initial potency or subsequent loss of potency [8], drugs inhibiting the allergic reaction, diseases attenuating the skin response, decreased skin reactivity in infants and elderly patients, improper technique (no or weak puncture). The use of positive control solutions may overcome some of the false-negative results because reactions will be either decreased or inhibited in patients with poorly reactive skin.

The occurrence of positive responses to skin tests does not necessarily imply that the patient's symptoms are due to an IgE-mediated allergy, since skin-prick tests are positive in 15–35% of symptom-free individuals depending on the allergen and the area (for review see [1]). The presence of positive skin tests in asymptomatic subjects may precede the onset of allergic symptoms [50, 51], especially if the allergen load is high.

Clinical Value of Skin Tests

Even after false-positive and false-negative tests have been eliminated, the proper interpretation of results requires a thorough knowledge of the history and the physical findings. A positive skin test alone does not necessarily confirm a definite clinical reactivity to an allergen.

With inhalant allergens, skin test responses represent one of the first-line diagnostic methods and when they correlate with the clinical history, in vitro tests may not be required [12, 13, 52].

For foods, particular caution should be used, since very few extracts are standardised and some may be insufficiently sensitive. Extracts made from fruits and vegetables are usually of poor quality, since the allergens are rapidly destroyed. Skin tests with fresh foods are more sensitive [1]. Recombinant allergens will certainly improve the diagnosis of food allergy.

For occupational rhinitis, skin tests are often unreliable, except in the case of high-molecular-weight compounds such as latex or grain dust.

Nasal Challenge Tests

Nasal challenge tests are used in research and to a lesser extent in clinical practice, but they are particularly important in the diagnosis of occupational rhinitis.

Recommendations on and critical analysis of nasal provocations and methods to measure the effects of such tests have already been published by a subcommittee of the 'International Committee on Objective Assessment of the Nasal Airways' [53–55] (Table 1), which has devised guidelines for nasal provocation tests concerning indications, techniques and evaluation of the tests.

Methods

Different methods for the provocation and measurement of nasal responses have been used. Each technique has its own advantages and disadvantages. For clinical purposes, techniques for qualitative measurements may be appropriate, but for experimental research, quantitative measurements with high reproducibility are essential [56] (Table 2).

Table 1 Indications for nasal challenge tests [54]

1. Allergen provocations:
- When discrepancies between history of allergic rhinitis and tests or between tests are present (e.g. in cases of diagnostic doubt).
- For diagnosis of occupational allergic rhinitis.
- Before immunotherapy for allergic rhinitis. Although it is still not very common to use nasal provocation before starting immunotherapy, a laborious long-lasting therapy is justified by a proper diagnosis. This holds true particularly in the case of perennial allergic rhinitis.
- For research.

2. Lysine-aspirin: Nasal provocation is recommended as a substitute for oral provocation in aspirin hypersensitivity. Whenever such nasal provocation is negative, an oral test is still required.

3. To test non-specific hyperreactivity:
- Nasal provocation with non-specific stimuli (histamine, methacholine, cold dry air, etc.) is not relevant for daily clinical practice and diagnosis, but can be used in research.

Table 2 Recommendations for the performance of nasal challenge tests [54]

1. Provoking agent
- Use solutions at room temperature
- Standardised extracts
- Isotonic solutions buffered to a pH of about 7
- Use control solutions

2. Administration into the nose
- Metre-dose pump spray
- Paper disks

3. Assessment of the nasal response: symptom scores are combined with objective measures
- Counting sneezes or attacks of sneezing
- Measuring volume or weight of nasal secretion
- Changes of nasal patency, airflow or airflow resistance

4. Methods to evaluate nasal patency, airflow and airflow resistance
- Rhinomanometry
- Acoustic rhinometry
- Rhinostereometry
- Nasal inspiratory or expiratory peak flow

Less common methods are:
- Head-out body plethysmography
- Oscillometry

Provoking Agents

Allergens are usually administered in an aqueous solution, but although the solution is easy to administer into the nostrils, this form of challenge has many limits:

- Allergen extracts are not always standardised. Only standardised ones should be used when available.
- Allergen extracts may not represent the native allergen and the amount of allergen administered is far greater than that entering the nose during natural allergen

exposure. This is also true for recombinant allergens. As an example, although rBet v 1 alone is sufficient for a reliable diagnosis of birch pollen allergy in most patients and induces skin test reactivity comparable to that of birch pollen extract, it provokes fewer allergic reactions in nasal provocations [57].
- The potency of an aqueous extract often decreases rapidly and it is advisable, at least for research projects, to use standardised and lyophilised extracts of the same batch freshly reconstituted on the day of the test.
- Preservatives such as glycerol, benzalkonium chloride and phenol can induce non-specific nasal reactions.
- Temperature, pH and osmolarity of the solution should be checked carefully.

Allergens can also be administered in the form of a powder [58], as a solution adsorbed on a paper disk, or in the form of pollen grains mixed with lactose in capsules.

Administration in the Nose

Aqueous allergen extracts can be delivered from atomisers and an exact dose can be applied. Other investigators use a pipette and allergens are deposited during rhinoscopy. When using any of these methods, care should be taken to avoid non-specific responses, and for all experiments, the diluent of the allergen extract must be administered before the allergen, to test for the non-specific response. Small paper disks can be directly applied to the nostrils and allergen powders or pollen grains can be insufflated easily with Spinhalers or derived devices.

Other methods are of interest. In the Vienna Challenge Chamber [59] or the environmental exposure unit [60], patients are challenged under controlled conditions with purified airborne grass pollen. However, these conditions are only used for large clinical trials and are not useful in the diagnosis of allergic rhinitis.

Assessment of the Response

Different methods have been used to assess the response to allergens. None of these are fully accepted by all investigators.

Symptoms produced after a challenge can be recorded. Sneezing, rhinorrhea and nasal blockage are easy to assess and yield valuable information. However, patients can react with different symptoms on different test days and it is preferable to record more than one symptom [61]. Nasal obstruction is one of the cardinal symptoms in allergic rhinitis and the major symptom of the late-phase reaction following allergen challenges. The objective measurement of this symptom is therefore of greatest importance. However, physiological fluctuations in nasal resistance may be a problem in monitoring the nasal provocation test [62].

So far, *rhinomanometry* is the best evaluated and standardised technique [53, 63]. Active anterior rhinomanometry was recommended by an international committee

in 1984 [53]. Unlike active posterior rhinomanometry, with active anterior rhinomanometry, unilateral measurements can be made.

Acoustic rhinometry [64], characterised by a low coefficient of variation, has been used in nasal challenge tests with bradykinin, histamine and allergens. It appears to be a safe, non-invasive, objective and validated measure of nasal obstruction. However, acoustic rhinometry has limitations and pitfalls. The value of acoustic rhinometry in evaluating nasal responses after provocation in routine clinical work is not yet established although it is gaining more importance [65].

Rhinostereometry [66] can be used to record changes in the thickness of the nasal mucosa. With a microscope, 0.2 mm changes can be recorded in test subjects fixed to the apparatus using an individually made plastic splint adapted to the teeth. Rhinostereometry is, however, a time-consuming method. It seems useful for comparisons between well-defined groups of subjects and between the same subjects on different occasions. It may be combined with laser Doppler flowmetry [67].

Nasal peak flow appears to correlate very well with rhinomanometry [68].

Other methods, such as *rhinostereometry* and *whole body plethysmography*, have been proposed [69], but they have not yet been fully assessed.

Comparisons between methods are also available. In order to find the most sensitive method, assessments were made by means of symptom score, acoustic rhinometry, nasal peak expiratory and inspiratory flow, and rhinomanometry during histamine challenges [70]. There was no difference in the mucosal reactivity between patients and controls, regardless of the method used, but nasal peak flows were more sensitive to mucosal changes than the other methods studied.

Measurement of Mediators and Cells during Challenges

Allergen-specific nasal challenges are a valid and reliable tool for studying the pathophysiological mechanisms involved in allergic inflammation. Nasal challenges induce an immediate and late response in allergic subjects with the release of pro-inflammatory mediators. Nasal biopsies may also be obtained and have been used in many drug trials [71]. Measurement of mediators in the nose may increase the sensitivity of nasal challenges [72], but more data are needed.

Factors Affecting Nasal Challenges

As in any other in vivo tests, the major factors affecting nasal challenges are the quality of the allergens used and the drugs taken by the patient. Other factors are more specific to nasal challenges, including technical problems already discussed and inflammation of the nasal mucosa.

Sodium cromoglycate should be withdrawn 48 h before the test, the second generation H1-antihistamines and nasal steroids 5–6 days before, ketotifen and

imipramines 2 weeks before, and astemizole at least 1 month before. Nasal vasoconstrictors may not modify nasal challenges. Specific immunotherapy decreases the sensitivity of the nose to allergens.

The nasal mucosa may be altered by several factors and the response to allergens may be greatly affected. Indeed, it has been shown that an allergic reaction significantly increases the reactivity of the nose following a subsequent stimulation because of the priming effect initially described by Connell [73]. Viral infections, pollutants induce the release of pro-inflammatory mediators and cytokines in nasal secretions. Nasal challenges should thus be performed at least 2–4 weeks after any allergic or infectious episode. Finally, the nasal cycle [62] should be taken into consideration when rhinomanometry is used.

Challenges with Occupational Agents

The diagnosis of occupational rhinitis is often complex and requires nasal provocation tests with the relevant occupational agent [74–76]. The challenge can be carried out in the form of natural exposure, especially if the relevant allergen is unavailable. For example, this has been done for laboratory animal allergy in a vivarium during cage cleaning [76].

Bronchial Challenge Tests

Asthma is a multifactorial disease in which the non-specific bronchial hyperreactivity is a key factor, and so it is sometimes difficult to determine the exact importance of allergy. However, bronchial challenge tests with allergens are mainly used in research and to a lesser extent in clinical practice maybe with the exception of occupational asthma. Although easier to carry out, nasal or conjunctival provocation tests cannot replace bronchial challenge tests [77].

Technique

The traditional method for performing bronchial challenges is to use allergens in a solution form administered by nebulisers or in chambers, the patient handling the suspected allergen. Usually, the response is measured using FEV_1 and a provocative dose inducing a 20% reduction from the baseline is required to classify a bronchial challenge as positive ($PD_{20}FEV_1$) [78, 79].

Provoking Agents

The vast majority of inhaled allergens are in the form of aqueous solutions similar to those used for nasal challenges. Because most asthmatics have increased bronchial hyperreactivity, inhaled solutions must be isotonic, iso-osmolar and close to

body temperature and without irritant preservatives [80]. In research trials, it is recommended to use standardised lyophilised extracts of the same batch reconstituted on each of the test days.

However, administration of nebulised extracts bears little resemblance to natural allergen exposure in terms of allergen form, size, dose and site of deposition in the airways. In the bronchial provocation test, enormous doses of 1–4 µm droplets inhaled through the mouth reach all parts of the airways [80]. During natural exposure, the 20–60 µm pollen grains, animal allergens, or mite faeces are trapped mostly in the nose. Daily exposure consists of thousands of microprovocations of very small amounts of allergen. In case of animal dander or exposure to mould, the size of the particles is smaller (1–5 µm). However, aerosol challenges are still widely used and attempts have been made to standardise the methods.

Since severe asthmatic reactions may occur during allergen challenges, increasing doses of allergens are administered until a positive immediate reaction demonstrates that the maximum tolerable dose has been administered.

Natural allergen exposures are made in environment challenge exposure chambers, which are discussed below.

Administration into the Airways

The administration of allergens into the airways depends on many factors, including the aerodynamic size of the particles [80]. Two methods have gained more acceptance than others and provide remarkably similar results: intermittent generation of aerosols during full deep inspiration or continuous aerosol generation during tidal volume [81]. However, there are more sophisticated methods available, such as computerised equipment for the delivery of inhaled doses of solid particles in specific bronchial challenges [82].

Assessment of the Response

Many different techniques are also available. It is now accepted that the test should be stopped when an immediate response is observed with at least a 20% fall in FEV1 ($PD_{20}FEV_1$), a 25% fall in the maximum mid-expiratory flow rate, or a 35% increase in specific airway resistances [78, 80]. However, the most used is $PD_{20}FEV_1$ since FEV_1 is the most reproducible lung function test [83]. In some cases, $PD_{15}FEV_1$ is used, making it advisable to measure the pulmonary function serially to monitor late-phase reactions, if they occur.

Early- and Late-Phase Reactions

The typical reaction to inhaled allergens is characterised by an early (immediate) airway response that fully develops within 10–20 min and is rapidly reversible after inhalation of a short-acting β2 agonist. This reaction is sometimes followed by a

late-phase reaction that develops within 3–5 h after the challenge and usually peaks at 6–10 h. In some patients, these reactions are followed for several days, usually in the morning, by episodes of bronchoconstriction.

The early-phase reaction is typically due to bronchospasm, whereas the late-phase reaction is associated with airway inflammation.

Reproducibility of the Response

Many studies have confirmed that asthmatic responses induced by allergen challenges have a good reproducibility [84]. This is the reason for the widespread use of bronchial challenges in the development of drugs.

Factors Affecting Bronchial Challenges

The response to inhaled allergens is determined by the level of allergenic sensitivity as well as by the level of non-specific airway hyper-responsiveness [85, 86].

As in all in vivo tests, the major factors affecting nasal challenges are the quality of both the allergens used and the drugs taken by the patient. There are also other factors more specific to bronchial challenges, including baseline airway calibre, viral infections, recent asthma exacerbations and pollutants (in particular tobacco smoke).

It is usually recommended to avoid allergen challenges if the baseline FEV_1 is under 70% of predicted values, because the reaction induced by the inhaled allergen may lead to a severe asthmatic reaction [79].

Use of short-acting inhaled β2-agonists, aspirin, theophylline or anticholinergics should be stopped for at least 12 h prior to the test. Long-acting β2 agonists should be stopped for 24 h. Cromoglycate and inhaled corticosteroids should be stopped for a week.

Environmental Exposure Units

Pollen exposure in the environmental exposure unit is an effective, reproducible, safe and suitable method for single-centre clinical studies [59, 60]. These exposure units are mostly used to assess the efficacy of anti-allergic treatments. However, the priming effect [73] on the nasal mucosa is not considered in many studies and the results of the challenges do not usually accord with clinical data.

In cat allergy, exposure to cats in environmental exposure units has been widely used [87], but there is a high variability of cat allergen during these studies.

There are also environmental exposure units that are used for the diagnosis of occupational allergy. These are of great value and have been used for latex sensitisation [88].

Conjunctival Challenge Tests

The conjunctival challenge test is useful in clinical research. When indicated in clinical routine, it is a safe and easy test with good precision [89–91]. Nasal and conjunctival challenge tests give similar results in many, but not all patients [92]. Occupational allergy can also be determined using conjunctival challenges [93].

Technique

Provoking Agents

Allergens are administered in an aqueous solution.

Administration in the Eye

Conjunctival provocation tests are usually performed by applying 20 µl of the diluent and then 20 µl of the allergen solution in the conjunctival cul-de-sac.

Assessment of the Response

The positivity of conjunctival provocation tests can be assessed by symptom score or by the release of mast cell-derived mediators. Abelson proposed an easy scoring system [91] (Table 3). Conjunctival provocation tests is positive when the cumulative score is ≥ 5. For clinical purposes, only the early-phase reaction is recorded [94]. For research or pharmacologic purposes, both the early- and late-phase responses may be examined. Cells and inflammatory mediators can be assessed [95], but they are only useful in research and for assessing drug mechanisms [96]. Many studies have been performed using conjunctival challenges to assess the efficacy of oral [96, 97] or ocular drugs [98, 99] and specific immunotherapy [100].

More sophisticated methods can be used, such as digital imaging [101], scanning and imaging technology [102] or using a fractional millimetre reticule in the eyepiece of a slit lamp microscope for quantifying eyelid swelling [103], spectroradiometer or colorimeter for measuring erythema [103].

Factors Affecting Conjunctival Challenges

As for all in vivo tests, the major factors affecting conjunctival provocation tests are the quality of both the allergens used and the drugs taken by the patient. There are also other factors more specific to nasal challenges, including technical problems already discussed and inflammation of the nasal mucosa.

Table 3 Scoring system to measure the signs and symptoms of allergic conjunctivitis [91]

Redness, eyelid swelling
0: None
1: Mild
2: Moderate
3: Severe
Chemosis
0: None
1: Mild, detectable with slit lamp, conjunctiva separated from sclera
2: Moderate (visually evident, raised conjunctiva, especially in the limbal area)
3: Severe (ballooning of conjunctiva)
Tearing
0: None
1: Mild (eyes feel slightly watery)
2: Moderate (blows nose occasionally)
3: Severe (tears rolling down cheeks)
Itching (to be graded by subject)
0: None
1: Mild (intermittent tickling sensation)
2: Moderate (continual awareness but without the desire to rub)
3: Severe (continual awareness with the desire to rub the eyes)
4: Incapaciting itching (subject insists on rubbing eyes)

Clinical Value and Indication for Conjunctival Challenges

The results of conjunctival provocation tests appear to be more reproducible than those of nasal challenges, but vary more than the results of skin tests or IgE measurement. The challenge is time-consuming and may be unpleasant, and so it is not routinely used. Conjunctival provocation tests, however, may be used in clinical practice to confirm the allergy of a patient, especially when there is a discrepancy between the results of skin and in vitro tests or when immunotherapy is indicated. They can also be used to assess the results of specific immunotherapy or drug trials.

Laboratory Tests

The diagnosis of IgE-mediated hypersensitivity is based on positive histories, skin tests and/or specific-IgE assays. Sometimes identification of the causal factors is not easy because of the high number of allergens and the fact that not all allergies are IgE-dependent. In vitro tests are of great interest to allergists in order to establish the culprit agent and to avoid provocations, especially for patients exposed to several allergens simultaneously or with histories of life-threatening reactions.

Serum-Specific IgE Assays

The discovery of IgE in 1967 was a major advance in the understanding and diagnosis of allergic diseases [104, 105]. In normal subjects, levels of IgE increase from birth (0–1 KU/l) to adolescence and then decrease slowly and reach a plateau after the age of 20–30 years. In adults, levels of over 100–150 KU/l are considered to be above normal. Allergic and parasitic diseases as well as many other conditions increase the levels of total IgE in serum. Thus, the measurement of total serum IgE is barely predictive for allergy screening and should no longer be used as a diagnostic tool.

In contrast to the low predictive value of total serum IgE measurements in the diagnosis of immediate type allergy, the measurement of allergen-specific IgE in serum is of importance.

Methods

The first technique used to accurately measure serum specific IgE was the RAST (radioallergosorbent test) [106]. New techniques are now available using either radio- or enzyme-labelled anti-IgE [107, 108]. The different reagents are critical for an appropriate assay. Results are expressed in terms of total radioactive counts bound (cpm), arbitrary units (RAST class, PRU/ml) or units of IgE (IU/ml, KU/l).

Factors Affecting the Measurement of Serum Specific IgE

Specific IgE measurements are not influenced by drugs or skin diseases. Many technical factors can affect the measurement of IgE. The quality of reagents used (allergens, anti-IgE antibodies) is of importance. IgE antibody assays need to be sensitive and specific to make quantitative measurements over as wide a range as possible [109]. A high-capacity solid phase provides a large excess of allergen that maximises the binding of IgE antibody. The anti-IgE preparations applied must be Fcε-specific and preferably combinations of monoclonal antibodies with specificities against more than one epitope on the Fc fragment and with complementary dose-response characteristics. Calibrators should be traceable to the WHO International Reference Preparation for human IgE, 75/502 [110].

As for skin tests, the quality of allergens is of critical importance and, when possible, only standardised extracts should be used. Standardisation of the allergen source material in combination with adequate reagent design provides precise and reproducible data increasing the accuracy and efficiency of allergy diagnostic testing. However, using molecular biology, it is possible to obtain large quantities of major allergens for many species. Recombinant Bet v I produced in bacterial expression systems allows accurate in vitro diagnosis of birch pollen allergy in over

95% of birch pollen allergic patients [111]. Other studies have found similar values for recombinant allergens. Thus, single recombinant allergen or a combination of a few major recombinant allergens or the addition of some relevant recombinant allergens to an allergen extract can substitute the crude extract for in vitro diagnostic purposes [112]. It also seems that in vitro diagnostics for pollen allergy can be simplified using cross-reactivities. Current diagnostic extracts for grass pollen allergy are usually composed of mixtures of pollen from different grass species. Their complex composition hampers accurate standardisation. It was recently shown that the use of one grass species is sufficient for the in vitro diagnosis of grass pollen allergy. Purified natural Lol p 1 and Lol p 5 detect over 90% of grass-positive patients. Around 80% of the IgE response to grass pollen is directed to these major allergens [113]. The same is true for purified natural Bet v 1, Bet v 2 and profiling to diagnose Fagales pollen allergy [114].

Significance of Measurement of Serum Allergen Specific IgE

Several studies have shown that serum-specific IgE results correlate closely to those of skin tests and nasal challenges. As in skin tests, the presence or absence of specific IgE in the serum does not preclude symptoms, and some symptom-free subjects have serum-specific IgE. Although a low specific IgE titre may not be clinically relevant, the titre of serum-specific IgE is usually unrelated with symptoms. This is because the severity of symptoms depends not only on IgE antibodies but also on the releasability of mediators, the response of the target organ to mediators and non-specific hypersensitivity. When using single allergen tests, the cost of serum-specific IgE measurement is high and only a selected list of allergens can usually be tested.

Screening Tests Using Serum Specific IgE

Some methods use either a mixture of several allergens in a single assay [115] or test several different allergens during a single assay. These tests can therefore be used by specialised doctors and non-allergists as screening tests for the diagnosis of allergic diseases.

The clinical relevance of these tests has been extensively studied and it has been shown that their efficiency (specificity and sensitivity) in allergy diagnosis is often over 85% [115]. However, using these tests, the patient is defined only as allergic or non-allergic and more extensive investigations for rhinitis are needed if the test is positive.

Basophil Activation Tests

Basophils are major effector cells of allergic disease; they express the high-affinity IgE receptor (FcεRI), which is the main activating receptor for these cells. Flow cytometry has been used to investigate activation of human

basophils, both in vitro and in vivo [116], and it gives information on anaphylactic reactions.

The main advantages of the latest-generation flow cytometry analysers are the capability of identifying cells even in very small percentages of the total population examined (below 1%). In patients with immediateallergic reactions, a flow cytometric basophil activation test to detect specific surface markers with monoclonal antibodies can also be performed. At present, the most commonly used antigens in basophil activation tests are CD63 and CD203c. However, a new basophil identification antigen, CRTH2 (chemoattractant receptor-homologous molecule expressed on T-helper 2 cells) and the recently described activation antigens CD13, CD164 (behaving as CD203c), and CD107a (paralleling CD63 expression) could be applied in order to improve it [117].

However, the assessment of basophil activation as a clinical tool for the diagnosis of allergic disease is still in its infancy. Cytometry-assisted investigation of human basophils has been used to evaluate patients sensitised to inhalant allergens [118, 119], food [120, 121], latex [122, 123], drugs [124–127], and insect venom [128, 129], and is also helpful to study patients with autoimmune chronic urticaria [130, 131].

Microarrays

The microarray is an ordered array of microscopic elements on a planar substrate that allows the specific binding of genes or proteins [132, 133]. Protein microarrays are suitable to study protein–ligand interactions in which the ligand can be a protein, peptide, DNA, RNA, oligosaccharide or chemical compound. Any microarray assay provides a precise measure of the number, amount or concentration of the molecules present in a sample. The feasibility of using allergen-chips or arrayed allergens for multiallergen testing has been reported [134, 135]. In contrast to conventional allergy diagnosis, allergen microarray permits the simultaneous investigation of several hundred allergens and the measurement of different classes of immunoglobulin (IgE/IgG4) in a single step, and requires only a small amount of serum.

Microarrays were first used in the field of allergic diseases to analyse recombinant allergens to determine the individual sensitisation profile of patients [136]. DNA and protein microarrays have then been assessed for the diagnosis of respiratory allergy, and particularly bronchial asthma and rhinitis, atopic dermatitis, food allergy, and hypersensitivity to aspirin [137–141].

Microarray technologies have already proven to be invaluable in the field of genomics and proteomics and solutions to these challenges will undoubtedly facilitate the development of clinically useful diagnostic chips in the foreseeable future.

Clinical Value of the Tests and Correlation Between Tests

The diagnosis of allergy is based on the correlation between the clinical history and diagnostic tests for allergy. It is not possible to diagnose allergy based solely on responses to skin tests, in vitro tests, or even challenges. Factors affecting tests should always be checked before investigations, since drug therapy may modify the results of in vivo tests for days or even weeks. The importance of allergen challenges depends on the disease and the quality of the allergen extract considered. The different tests used in the diagnosis of allergy do not have the same biological and clinical significance.

Diagnosis of Inhalant Allergy

Skin tests represent the primary diagnostic tools used for immediate-type hypersensitivity. Comparisons between the measurement of specific IgE and skin tests depend on the quality and standardisation of the allergens used in both types of tests and, to a lesser extent, on the method of skin testing used. The weakest correlations have been obtained with mould and unstandardised dander extracts. For standardised allergens, challenges are usually not necessary to confirm the diagnosis of allergy. There are good correlations between a strongly positive response to a skin test and the detection of serum-specific IgE and between a negative response to a prick test and the lack of detection of serum-specific IgE, whereas small wheals induced by prick tests and positive results of intradermal tests with concentrated extracts are less frequently associated with the detection of serum-specific IgE. Positive responses to skin tests and serum-specific IgE can be found in totally symptom-free subjects with a similar prevalence.

Correlations between responses to skin tests and to the measurement of allergen-specific IgE with inhalation challenges are less consistent because of the non-specific hyper-reactivity. Poor correlations are observed with un-standardised extracts, weakly positive responses to skin testing, and the RAST, or when there is a discrepancy between the clinical history and skin tests. When there are both a suggestive history and strongly positive responses to skin testing and the RAST, inhalation challenges are often positive. The prevalence of positive responses to inhalation challenges in symptom-free patients is lower than that of positive responses to skin testing or the RAST, but pollen-allergic patients who suffer only from rhinitis often manifest a positive response to bronchial challenge with pollen extracts.

Correlations between responses to skin tests and serum-specific IgE with nasal challenges are less consistent because of the non-specific nasal hyper-reactivity. Poor correlations are observed with un-standardised extracts, weak positive responses to skin testing, and the RAST, or when there is a discrepancy between the clinical history and skin tests.

Before the discovery of IgE and when poorly characterised allergens were used, false-negative results were frequent and the bronchial challenge was useful to diagnose allergic asthma. It is now mostly restricted to research protocols, assessment of drugs in development, and occupational medicine.

Correlations have not been performed with environmental exposure chambers or conjunctival challenges.

Diagnosis of Food Allergy

While allergic reactions to foods are usually due to IgE-mediated hypersensitivity mechanisms, there are a number of immune mechanisms that may contribute to adverse reactions to foods. Tests for allergic reactions include both skin-prick tests and the measurement of serum allergen-specific IgE antibodies. The diagnosis of food allergy is complicated, however, because allergen extracts and the test reagents currently available are not standardised and their stability is poorly determined. The presence of food-specific IgE in serum or a positive skin test to a foodstuff does not always correlate with a food allergy since some patients outgrow their allergy with age and not all patients with food-specific IgE have a clinical sensitivity. In many instances, the diagnosis has to be confirmed by a double-blind food challenge, which should be carried out under precisely specified conditions and by staff who have the competence to manage anaphylactic reactions. As for other forms of allergy, unproven and controversial techniques such as cytotoxic tests, VEGA testing or sublingual provocation tests have no proven value.

Diagnosis of Occupational Allergy

Occupational rhinitis must be more precisely confirmed than allergic rhinitis of other aetiology. In practice, interviews concerning the causal relation, frequency, latent period and atopic disposition often provide suggestions, but sometimes give unreliable evidence for diagnosing occupational nasal allergy. Therefore, examinations such as skin tests, nasal provocation tests and determination of the IgE antibody level are necessary to confirm the causality between the disease and the work exposure.

References

1. Demoly P, Piette V, Bousquet J (2003) In vivo methods for study of allergy: skin tests, techniques and interpretation. In: Adkinson NF Jr, Yunginger JW, Busse WW, Bochner BS, Holgate ST, Simons FER (eds). Allergy, Principles and Practice, 6th Edition. Mosby, New York, pp. 631–655
2. Pepys J (1975) Skin testing. Br J Hosp Med 14:412
3. Osterballe O, Weeke B (1979) A new lancet for skin prick testing. Allergy 34:209–212

4. Perrin LF, Dechamp C, Deviller P, Joly P (1984) Reproducibility of skin tests. A comparative study of the Pepys prick test and the Morrow-Brown needle and their correlation with the serum IgE level. Clin Allergy 14:581–588
5. Basomba A, Sastre A, Pelaez A, Romar A, Campos A, Garcia-Villalmanzo A (1985) Standardization of the prick test. A comparative study of three methods. Allergy 40:395–399
6. Demoly P, Bousquet J, Manderscheid JC, Dreborg S, Dhivert H, Michel FB (1991) Precision of skin prick and puncture tests with nine methods. J Allergy Clin Immunol 88:758–762
7. Nelson HS, Rosloniec DM, McCall LI, Ikle D (1993) Comparative performance of five commercial prick skin test devices. J Allergy Clin Immunol 92:750–756
8. Dreborg S, Backman A, Basomba A, Bousquet J, Dieges P, Malling H (1989) Skin tests used in type I allergy testing. Position paper of the European Academy of Allergy and Clinical Immunology. Allergy 44:S1–69
9. Reid MJ, Lockey RF, Turkeltaub PC, Platts-Mills TA (1993) Survey of fatalities from skin testing and immunotherapy 1985–1989. J Allergy Clin Immunol 92:6–15
10. Nelson HS, Oppenheimer J, Buchmeier A, Kordash TR, Freshwater LL (1996) An assessment of the role of intradermal skin testing in the diagnosis of clinically relevant allergy to timothy grass. J Allergy Clin Immunol 97:1193–1201
11. Wood RA, Phipatanakul W, Hamilton RG, Eggleston PA (1999) A comparison of skin prick tests, intradermal skin tests, and RASTs in the diagnosis of cat allergy. J Allergy Clin Immunol 103:773–779
12. Bernstein IL, Storms WW (1995) Practice parameters for allergy diagnostic testing. Joint Task Force on Practice Parameters for the Diagnosis and Treatment of Asthma. The American Academy of Allergy, Asthma and Immunology and the American College of Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol 75:543–625
13. The European Academy of Allergology and Clinical Immunology (1993) Position paper: Allergen standardization and skin tests. Allergy 48:S48–S82
14. Board of Directors. American Academy of Allergy and Immunology (1993) Allergen skin testing. J Allergy Clin Immunol 92:636–637
15. Romano A, Di Fonso M, Giuffreda F, Quaratino D, Papa G, Palmieri V, Zeppilli P, Venuti A (1995) Diagnostic work-up for food-dependent, exercise-induced anaphylaxis. Allergy 50:817–824
16. Hansen TK, Host A, Bindslev-Jensen C (2004) An evaluation of the diagnostic value of different skin tests with egg in clinically egg-allergic children having atopic dermatitis. Pediatr Allergy Immunol 15:428–434
17. Seidenari S, Manzini BM, Danese P (1992) Patch testing with pollens of Gramineae in patients with atopic dermatitis and mucosal atopy. Contact Dermatitis 27:125–126
18. Darsow U, Vieluf D, Ring J (1995) Atopy patch test with different vehicles and allergen concentrations: an approach to standardization. J Allergy Clin Immunol 95:677–6784
19. Ring J, Darsow U, Gfesser M, Vieluf D (1997) The 'atopy patch test' in evaluating the role of aeroallergens in atopic eczema. Int Arch Allergy Immunol 113:379–383
20. Heine RG, Verstege A, Mehl A, Staden U, Rolinck-Werninghaus C, Niggemann B (2006) Proposal for a standardized interpretation of the atopy patch test in children with atopic dermatitis and suspected food allergy. Pediatr Allergy Immunol 17:213–217.
21. Turjanmaa K, Darsow U, Niggemann B, Rance F, Vanto T, Werfel T (2006) EAACI/GA2LEN position paper: present status of the atopy patch test. Allergy 61:1377–1384
22. Breuer K, Heratizadeh A, Wulf A, Baumann U, Constien A, Tetau D, Kapp A, Werfel T (2004) Late eczematous reactions to food in children with atopic dermatitis. Clin Exp Allergy 34:817–824
23. Darsow U, Laifaoui J, Kerschenlohr K, Wollenberg A, Przybilla B, Wüthrich B, Borelli S, Giusti F, Seidenari S, Drzimalla K, Simon D, Disch R, Borelli S, Devillers AC, Oranje AP, De Raeve L, Hachem JP, Dangoisse C, Blondeel A, Song M, Breuer K, Wulf A, Werfel T, Roul S, Taieb A, Bolhaar S, Bruijnzeel-Koomen C, Brönnimann M, Braathen LR, Didierlaurent A, André C, Ring J (2004) The prevalence of positive reactions in the atopy

patch test with aeroallergens and food allergens in subjects with atopic eczema: a European multicenter study. Allergy 59:1318–1325
24. Malling HJ (1984) Skin prick testing and the use of histamine references. Allergy 39:596–601
25. Bousquet J, Djoukadar F, Hewitt B, Guerin B, Michel FB (1985) Comparison of the stability of a mite and a pollen extract stored in normal conditions of use. Clin Allergy 15:29–35
26. Scherer K, Grize L, Schindler C, Surber C, Bircher AJ (2007) Reaction pattern to histamine and codeine in a human intradermal skin test model. Clin Exp Allergy 37:39–46
27. Valenta R, Vrtala S, Ebner C, Kraft D, Scheiner O (1992) Diagnosis of grass pollen allergy with recombinant timothy grass (*Phleum pratense*) pollen allergens. Int Arch Allergy Immunol 97:287–294
28. Moser M, Crameri R, Menz G, Schneider T, Dudler T, Virchow C, Gmachl M, Blaser K, Suter M (1992) Cloning and expression of recombinant Aspergillus fumigatus allergen I/a (rAsp f I/a) with IgE binding and type I skin test activity. J Immunol 149:454–460
29. Lynch NR, Thomas WR, Chua Y, Garcia N, Di Prisco MC, Lopez R (1994) In vivo biological activity of recombinant Der p II allergen of house-dust mite. Int Arch Allergy Immunol 105:70–74
30. Muller U, Fricker M, Wymann D, Blaser K, Crameri R (1997) Increased specificity of diagnostic tests with recombinant major bee venom allergen phospholipase A2. Clin Exp Allergy 27:915–920
31. Yip L, Hickey V, Wagner B, Liss G, Slater J, Breiteneder H, Sussman G, Beezhold D (2000) Skin prick test reactivity to recombinant latex allergens. Int Arch Allergy Immunol 121:292–299
32. Laffer S, Spitzauer S, Susani M, Pairleitner H, Schweiger C, Gronlund H, Menz G, Pauli G, Ishii T, Nolte H, Ebner C, Sehon AH, Kraft D, Eichler HG, Valenta R (1996) Comparison of recombinant timothy grass pollen allergens with natural extract for diagnosis of grass pollen allergy in different populations. J Allergy Clin Immunol 98:652–658
33. Pauli G, Oster JP, Deviller P, Heiss S, Bessot JC, Susani M, Ferreira F, Kraft D, Valenta R (1996) Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: diagnostic value for birch pollen and associated allergies. J Allergy Clin Immunol 97:1100–1109
34. Kusunoki T, Inoue Y, Korematsu S, Harazaki M, Yokota T, Hosoi S. (2000) Comparison of skin prick test with serially diluted wild-type and genetically engineered recombinant Der f2. Ann Allergy Asthma Immunol 84:366–368
35. Chapman MD, Smith AM, Vailes LD, Arruda LK (1997) Defined epitopes: in vivo and in vitro studies using recombinant allergens. Int Arch Allergy Immunol 113:102–104
36. Rossi RE, Monasterolo G, Operti D, Operti R, Berlen R (2000) Evaluation of IgE antibodies to recombinant pollen allergens (Phl p 1, Phl p 2, and Phl p 5) in a random sample of patients with specific IgE to Phleum pratense. Allergy 55:181–184
37. Mari A (2001) Multiple pollen sensitization: a molecular approach to the diagnosis. Int Arch Allergy Immunol 125:57–65
38. Bousquet J, Anto JM, Bachert C, Bousquet PJ, Colombo P, Crameri R, Daëron M, Fokkens W, Leynaert B, Lahoz C, Maurer M, Passalacqua G, Valenta R, van Hage M, Van Ree R (2006) Factors responsible for differences between asymptomatic subjects and patients presenting an IgE sensitization to allergens. A GA2LEN project. Allergy 61:671–680
39. Egger M, Mutschlechner S, Wopfner N, Gadermaier G, Briza P, Ferreira F (2006) Pollen-food syndromes associated with weed pollinosis: an update from the molecular point of view. Allergy 61:461–476
40. Hoffmann-Sommergruber K, Demoly P, Crameri R, Breiteneder H, Ebner C, Laimer Da Camara Machado M, Blaser K, Ismail C, Scheiner O, Bousquet J, Menz G (1999) IgE reactivity to Api g 1, a major celery allergen, in a Central European population is based on primary sensitization by Bet v 1. J Allergy Clin Immunol 104:478–484
41. Astier C, Morisset M, Roitel O, Codreanu F, Jacquenet S, Franck P, Ogier V, Petit N, Proust B, Moneret-Vautrin DA, Burks AW, Bihain B, Sampson HA, Kanny G (2006) Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. J Allergy Clin Immunol 118:250–256

42. Reuter A, Lidholm J, Andersson K, Ostling J, Lundberg M, Scheurer S, Enrique E, Cistero-Bahima A, San Miguel-Moncin M, Ballmer-Weber BK, Vieths S (2006) A critical assessment of allergen component-based in vitro diagnosis in cherry allergy across Europe. Clin Exp Allergy 36:815–823
43. Menardo JL, Bousquet J, Rodiere M, Astruc J, Michel FB (1985) Skin test reactivity in infancy. J Allergy Clin Immunol 75:646–651
44. Ownby DR, Adinoff AD (1994) The appropriate use of skin testing and allergen immunotherapy in young children. J Allergy Clin Immunol 94:662–665
45. Skassa-Brociek W, Manderscheid JC, Michel FB, Bousquet J (1987) Skin test reactivity to histamine from infancy to old age. J Allergy Clin Immunol 80:711–716
46. Oppenheimer JJ, Nelson HS (1993) Seasonal variation in immediate skin test reactions. Ann Allergy 71:227–229
47. Haahtela T, Jokela H (1980) Influence of the pollen season on immediate skin test reactivity to common allergens. Allergy 35:15–21
48. Bousquet J, Knani J, Hejjaoui A, Ferrando R, Cour P, Dhivert H, Michel FB (1993) Heterogeneity of atopy. I. Clinical and immunologic characteristics of patients allergic to cypress pollen. Allergy 48:183–188
49. Simons FE, Johnston L, Gu X, Simons KJ (2001) Suppression of the early and late cutaneous allergic responses using fexofenadine and montelukast. Ann Allergy Asthma Immunol 86:44–50
50. Jackson CM, Lee DK, Lipworth BJ (2004) The effects of butterbur on the histamine and allergen cutaneous response. Ann Allergy Asthma Immunol 92:250–254
51. Hagy G, Settipane G (1971) Prognosis of positive allergy skin tests in an asymptomatic population. A three year follow-up of college students. J Allergy 48:200
52. Horak F (1985) Manifestation of allergic rhinitis in latent-sensitized patients. A prospective study. Arch Otorhinolaryngol 242:239–245
53. Crobach MJ, Hermans J, Kaptein AA, Ridderikhoff J, Petri H, Mulder JD (1998) The diagnosis of allergic rhinitis: how to combine the medical history with the results of radioallergosorbent tests and skin prick tests. Scand J Prim Health Care 16:30–36
54. Clement PA (1984) Committee report on standardization of rhinomanometry. Rhinology 22:151–155
55. Malm L, Gerth-van-Wijk R, Bachert C (1999) Guildelines for nasal provocations with aspects on nasal patency, airflow, and airflow resistance. Rhinology 37:133–135
56. Andersson M, Greiff L, Svensson C, Persson C (1995) Various methods for testing nasal responses in vivo: a critical review. Acta Otolaryngol Stockh 115:705–713
57. Tresch S, Holzmann D, Baumann S, Blaser K, Wuthrich B, Crameri R, Schmid-Grendelmeier P (2003) In vitro and in vivo allergenicity of recombinant Bet v 1 compared to the reactivity of natural birch pollen extract. Clin Exp Allergy 33:1153–1158
58. Salzano FA (1997) Specific nasal provocation test with powder allergen. Allergy 52: 32–S35
59. Horak F, Jager S, Berger U (1992) Onset and duration of the effects of three antihistamines in current use – astemizole, loratadine and terfenadine forte – studied during prolonged, controlled allergen challenges in volunteers. J Int Med Res 20:422–434
60. Day JH, Briscoe M, Widlitz MD (1998) Cetirizine, loratadine, or placebo in subjects with seasonal allergic rhinitis: effects after controlled ragweed pollen challenge in an environmental exposure unit. J Allergy Clin Immunol 101:638–645
61. Lebel B, Bousquet J, Morel A, Chanal I, Godard P, Michel FB (1988) Correlation between symptoms and the threshold for release of mediators in nasal secretions during nasal challenge with grass-pollen grains. J Allergy Clin Immunol 82:869–877
62. Pirila T, Talvisara A, Alho OP, Oja H (1997) Physiological fluctuations in nasal resistance may interfere with nasal monitoring in the nasal provocation test. Acta Otolaryngol Stockh 117:596–600
63. Schumacher MJ (1989) Rhinomanometry. J Allergy Clin Immunol 83:711–718

64. Zweiman B, Getsy J, Kalenian M, Lane A, Schwartz LB, Doty R, Lanza D (1997) Nasal airway changes assessed by acoustic rhinometry and mediator release during immediate and late reactions to allergen challenge. J Allergy Clin Immunol 100:624–631
65. Wang DY, Raza MT, Goh DY, Lee BW, Chan YH (2004) Acoustic rhinometry in nasal allergen challenge study: which dimensional measures are meaningful? Clin Exp Allergy 34:1093–1098
66. Juto JE, Lundberg C (1982) An optical method for determining changes in mucosal congestion in the nose in man. Acta Otolaryngol 94:149–156
67. Grudemo H, Juto JE (1997) Rhinostereometry and laser Doppler flowmetry in human nasal mucosa: changes in congestion and microcirculation during intranasal histamine challenge. Orl J Otorhinolaryngol Relat Spec 59:50–56
68. Holmstrom M, Scadding GK, Lund VJ, Darby YC (1990) Assessment of nasal obstruction. A comparison between rhinomanometry and nasal inspiratory peak flow. Rhinology 28:191–196
69. de-Bruin-Weller MS, Weller FR, Scholte A, Rijssenbeek LH, van-der-Baan S, Bogaard JM, de Monchy JG (1996) Early and late allergic reaction in the nose assessed by whole body plethysmography. Eur Respir J 9:1701–1706
70. Scadding GK, Darby YC, Austin CE (1994) Acoustic rhinometry compared with anterior rhinomanometry in the assessment of the response to nasal allergen challenge. Clin Otolaryngol 19:451–454
71. Demoly P, Campbell A, Lebel B, Bousquet J (1999) Experimental models in rhinitis. Clin Exp Allergy 3:72–76
72. Raulf-Heimsoth M, Wirtz C, Papenfuss F, Baur X (2000) Nasal lavage mediator profile and cellular composition of nasal brushing material during latex challenge tests. Clin Exp Allergy 30:110–121
73. Connell JT (1969) Quantitative intranasal pollen challenges. 3. The priming effect in allergic rhinitis. J Allergy 43:33–44
74. Hytonen M, Sala E (1996) Nasal provocation test in the diagnostics of occupational allergic rhinitis. Rhinology 34:86–90
75. Palczynski C, Walusiak J, Ruta U, Gorski P (2000) Nasal provocation test in the diagnosis of natural rubber latex allergy. Allergy 55:34–41
76. Eggleston PA, Ansari AA, Adkinson N, Jr., Wood RA (1995) Environmental challenge studies in laboratory animal allergy. Effect of different airborne allergen concentrations. Am J Respir Crit Care Med 151:640–646
77. Stenius-Aarniala BS, Malmberg CH, Holopainen EE (1978) Relationship between the results of bronchial, nasal and conjunctival provocation tests in patients with asthma. Clin Allergy 8:403–409
78. Pepys J, Hutchcroft BJ (1975) Bronchial provocation tests in etiologic diagnosis and analysis of asthma. Am Rev Respir Dis 112:829–859
79. Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, Malo JL (1993) Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J 16:S53–S83
80. Fish J, Peters S (2003) Bronchial challenge testing. In: Adkinson N F Jr, Yunginger JW, Busse WW, Bochner BS, Holgate ST, Simons FER (eds) Middleton's Allergy Principles and Practice. 6th Edition. St Louis, Mosby, pp. 657–670
81. Ryan G, Dolovich MB, Roberts RS, Frith PA, Juniper EF, Hargreave FE, Newhouse MT (1981) Standardization of inhalation provocation tests: two techniques of aerosol generation and inhalation compared. Am Rev Respir Dis 123:195–199
82. Fabries JF, Choudat D, Wrobel R, Cloutier Y, Dessanges JF, Villette C, Dall Ava J, Conso F (2000) Computerized equipment for the delivery of inhaled doses of solid particles in specific bronchial challenge. J Aerosol Med 13:1–10

83. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, vane r Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J (2005) Interpretative strategies for lung function tests. Eur Respir J 26:948–968
84. Dente FL, Bacci E, di Franco A, Giannini D, Conti I, Macchioni P Scuoti L, Taccola M, Vagaggini B, Paggiaro PL (2000) Reproducibility of early and late asthmatic responses to allergen challenge in a large group of asthmatics. Respir Med 94:441–447
85. Bryant DH, Burns MW, Lazarus L (1975) The correlation between skin tests, bronchial provocation tests and the serum level of IgE specific for common allergens in patients with asthma. Clin Allergy 5:145–157
86. Cockcroft DW, Davis BE, Boulet LP, Deschesnes F, Gauvreau GM, O'Byrne PM, Watson RM (2005) The links between allergen skin test sensitivity, airway responsiveness and airway response to allergen. Allergy 60:56–59
87. Nelson H, Oppenheimer J, Vatsia G, Buchmeier A (1993) A double-blind, placebo-controlled evaluation of sublingual immunotherapy with standardized cat extract. J Allergy Clin Immunol 92:229–236
88. Kurtz KM, Hamilton RG, Schaefer JA, Primeau MN, Adkinson NF, Jr (2001) Repeated latex aeroallergen challenges employing a hooded exposure chamber: safety and reproducibility. Allergy 56:857–861
89. Moller C, Bjorksten B, Nilsson G, Dreborg S (1984) The precision of the conjunctival provocation test. Allergy 39:37–41
90. Bonini S, Berruto A, Tomassini M, Carlesimo S, Bucci MG, Balsano F (1989) Conjunctival provocation test as a model for the study of allergy and inflammation in humans. Int Arch Allergy Appl Immunol 88:144–148.
91. Abelson MB, Chambers WA, Smith LM (1990) Conjunctival allergen challenge. A clinical approach to studying allergic conjunctivitis. Arch Ophthalmol 108:84–88
92. Malmberg CH, Holopainen EE, Stenius-Aarniala BS (1978) Relationship between nasal and conjunctival tests in patients with allergic rhinitis. Clin Allergy 8:397–402
93. Alvarez MJ, Tabar AI, Quirce S, Olaguibel JM, Lizaso MT, Echechipia S, Rodriguez A, Garcia BE (1996) Diversity of allergens causing occupational asthma among cereal workers as demonstrated by exposure procedures. Clin Exp Allergy 26:147–153
94. Aichane A, Campbell AM, Chanal I, Richard MC, Arnaud B, Michel FB, Bousquet J (1993) Precision of conjunctival provocation tests in right and left eyes. J Allergy Clin Immunol 92:49–55
95. Bonini S, Vecchione A, Naim DM, Allansmith MR, Balsano F (1988) Inflammatory changes in conjunctival scrapings after allergen provocation in humans. J Allergy Clin Immunol 82:462–469
96. Ciprandi G, Buscaglia S, Pesce GP, Marchesi E, Canonica GW (1991) Protective effect of loratadine on specific conjunctival provocation test. Int Arch Allergy Appl Immunol 96:344–347
97. Ciprandi G, Buscaglia S, Iudice A, Pesce GP, Bagnasco M, Canonica GW (1993) Protective effects of deflazacort on allergen-specific conjunctival challenge. Eur J Clin Pharmacol 45:S35–S41
98. Abelson MB, Spitalny L (1998) Combined analysis of two studies using the conjunctival allergen challenge model to evaluate olopatadine hydrochloride, a new ophthalmic antiallergic agent with dual activity. Am J Ophthalmol 125:797–804
99. Persi L, Dupin O, Arnaud B, Trinquand C, Michel FB, Bousquet J (1997) Efficacy of mequitazine in comparison with placebo assessed by ocular challenge with allergen in allergic conjunctivitis. Allergy 52:451–454
100. Passalacqua G, Albano M, Fregonese L, Riccio A, Pronzato C, Mela GS, Canonica GW (1998) Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. Lancet 351:629–632
101. Horak F, Berger U, Menapace R, Schuster N (1996) Quantification of conjunctival vascular reaction by digital imaging. J Allergy Clin Immunol 98:495–500

102. Abelson MB, Pratt S, Mussoline JE, Townsend D (2003) One-visit, randomized, placebo-controlled, conjunctival allergen challenge study of scanning and imaging technology for objective quantification of eyelid swelling in the allergic reaction with contralateral use of olopatadine and artificial tears. Clin Ther 25:2070–2084
103. Friedlaender MH (2004) Objective measurement of allergic reactions in the eye. Curr Opin Allergy Clin Immunol 4:447–453
104. Ishizaka K, Ishizaka T (1967) Identification of gamma-E-antibodies as a carrier of reaginic activity. J Immunol 99:1187–1198
105. Johansson SG (1967) Raised levels of a new immunoglobulin class (IgND) in asthma. Lancet 2:951–953
106. Wide L, Bennich H, Johansson SG (1967) Diagnosis of allergy by an in-vitro test for allergen antibodies. Lancet 2:1105–1107
107. Bousquet J, Chanez P, Chanal I, Michel FB (1990) Comparison between RAST and Pharmacia CAP system: a new automated specific IgE assay. J Allergy Clin Immunol 85:1039–1043
108. van Houte AJ, Bartels PC (1992) Comparative evaluation of the pharmacia CAP system and the DPC AlaSTAT system for in vitro detection of allergen-specific IgE with the skin prick test. Eur J Clin Chem Clin Biochem 30:101–105
109. Bernstein I (1988) Proceedings of the Task Force on Guidelines for standardizing old and new technologies used for the diagnosis and treatment of allergic diseases. J Allergy Clin Immunol 82:487–526
110. Yman L (1991) Standardization of IgE antibody assays. J Int Fed Clin Chem 3:198–203
111. Menz G, Dolecek C, Schonheit-Kenn U, Ferreira F, Moser M, Schneider T, Suter M, Boltz-Nitulescu G, Ebner C, Kraft D, Valenta R (1996) Serological and skin-test diagnosis of birch pollen allergy with recombinant Bet v I, the major birch pollen allergen. Clin Exp Allergy 26:50–60
112. Olsen E, Mohapatra SS (1994) Recombinant allergens and diagnosis of grass pollen allergy. Ann Allergy 72:499–506
113. van Ree R, van Leeuwen WA, Aalberse RC (1998) How far can we simplify in vitro diagnostics for grass pollen allergy?: A study with 17 whole pollen extracts and purified natural and recombinant major allergens. J Allergy Clin Immunol 102:184–190
114. Van Ree R, Van Leeuwen WA, Akkerdaas JH, Aalberse RC (1999) How far can we simplify in vitro diagnostics for Fagales tree pollen allergy? A study with three whole pollen extracts and purified natural and recombinant allergens. Clin Exp Allergy 29:848–855
115. Eriksson NE (1990) Allergy screening with Phadiatop and CAP Phadiatop in combination with a questionnaire in adults with asthma and rhinitis. Allergy 45:285–292
116. Bochner BS (2000) Systemic activation of basophils and eosinophils: markers and consequences. J Allergy Clin Immunol 106:S292–S302
117. Ebo DG, Sainte-Laudy J, Bridts CH, Mertens CH, Hagendorens MM, Schuerwegh AJ, De Clerck CK, Stevens WJ (2006) Flow-assisted allergy diagnosis: current applications and future perspectives. Allergy 61:1028–1039
118. Sanz ML, Sanchez G, Gamboa PM, Vila L, Uasuf C, Chazot M, Diéguez I, De Weck AL (2001) Allergen-induced basophil activation: CD63 cell expression detected by flow cytometry in patients allergic to Dermatophagoides pteronyssinus and Lolium perenne. Clin Exp Allergy 31:1007–1013
119. Saporta M, Kamei S, Persi L, Bousquet J, Arnoux B (2001) Basophil activation during pollen season in patients monosensitized to grass pollens. Allergy 56:442–445
120. Moneret-Vautrin DA, Sainte-Laudy J, Kanny G, Fremont S (1999) Human basophil activation measured by CD63 expression and LTC4 release in IgE-mediated food allergy. Ann Allergy Asthma Immunol 82:33–40
121. Shreffler WG (2006) Evaluation of basophil activation in food allergy: present and future applications. Curr Opin Allergy Clin Immunol 6:226–233

122. Ebo DG, Lechkar B, Schuerwegh AJ, Bridts CH, De Clerck LS, Stevens WJ (2002) Validation of a two-color flow cytometric assay detecting in vitro basophil activation for the diagnosis of IgE mediated natural rubber latex allergy. Allergy 57:706–712
123. Hemery ML, Arnoux B, Dhivert-Donnadieu H, Rongier M, Barbotte E, Verdier R, Demoly P (2005) Confirmation of the diagnosis of natural rubber latex allergy by the Basotest method. Int Arch Allergy Immunol 136:53–57
124. Sanz ML, Gamboa PM, Antepara I, Uasuf C, Vila L, Garcia-Aviles C, Chazot M, De Weck AL (2002) Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. Clin Exp Allergy 32:277–286
125. Gamboa P, Sanz ML, Caballero MR, Urrutia I, Antepara I, Esparza R, de Weck AL (2004) The flow-cytometric determination of basophil activation induced by aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) is useful for in vitro diagnosis of the NSAID hypersensitivity syndrome. Clin Exp Allergy 34:1448–1457
126. Torres MJ, Padial A, Mayorga C, Fernandez T, Sanchez-Sabate E, Cornejo-Garcia JA, Antunez C, Blanca M (2004) The diagnostic interpretation of basophil activation test in immediate allergic reactions to betalactams. Clin Exp Allergy 34:1768–1775
127. Kvedariene V, Kamey S, Ryckwaert Y, Ronger M, Bousquet J, Demoly P, Arnoux B (2006) Diagnosis of neuromuscular blocking agent hypersensitivity reactions using cytofluorimetric analysis of basophils. Allergy 61:311–315
128. Sturm GJ, Bohm E, Trummer M, Weiglhofer I, Heinemann A, Aberer W (2004) The CD63 basophil activation test in Hymenoptera venom allergy: a prospective study. Allergy 59:1110–1117
129. Erdmann SM, Sachs B, Kwiecien R, Moll-Slodowy S, Sauer I, Merk HF (2004) The basophil activation test in wasp venom allergy: sensitivity, specificity and monitoring specific immunotherapy. Allergy 59:1102–1109
130. De Swerdt A, Van Den Keybus C, Kasran A, Cadot P, Neyens K, Coorevits L, Kochuyt AM, Degreef H, Ceuppens JL (2005) Detection of basophil-activating IgG autoantibodies in chronic idiopathic urticaria by induction of CD63. J Allergy Clin Immunol 116:662–667
131. Frezzolini A, Provini A, Teofoli P, Pomponi D, De Pità O (2006) Serum-induced basophil CD63 expression by means of a tricolour flow cytometric method for the in vitro diagnosis of chronic urticaria. Allergy 61:1071–1077
132. Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270: 467–470
133. MacBeath G, Schreiber SL (2000) Printing proteins as microarrays for high throughput function determination. Science 289:1760–1763
134. Suck R, Nandy A, Weber B, Stock M, Fiebig H, Cromwell O (2002) Rapid method for arrayed investigation of IgE-reactivity profiles using natural and recombinant allergens. Allergy 57:821–824
135. Harwanegg C, Laffer S, Hiller R, Mueller MW, Kraft D, Spitzauer S, Valenta R (2003) Microarrayed recombinant allergens for diagnosis of allergy. Clin Exp Allergy. 33:7–13
136. Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A, Barletta B, Becker WM, Blaser K, Breiteneder H, Chapman M, Crameri R, Duchêne M, Ferreira F, Fiebig H, Hoffmann-Sommergruber K, King TP, Kleber-Janke T, Kurup VP, Lehrer SB, Lidholm J, Müller U, Pini C, Reese G, Scheiner O, Scheynius A, Shen HD, Spitzauer S, Suck R, Swoboda I, Thomas W, Tinghino R, Van Hage-Hamsten M, Virtanen T, Kraft D, Müller MW, Valenta R. (2002) Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. FASEB J 16:414–416
137. Kim TE, Park SW, Cho NY Choi SY, Yong TS, Nahm BH, Lee S, Noh G (2002) Quantitative measurement of serum specific IgE on Protein Chip. Exp Mol Med 34:152–158
138. Lebrun SJ, Petchpud WN, Hui A, McLaughlin CS (2005) Development of a sensitive, colorometric microarray assay for allergen-responsive human IgE. J Immunol Methods 300:24–31

139. Shreffler WG, Lencer DA, Bardina L, Sampson HA (2005) IgE and IgG4 epitope mapping by microarray immunoassay reveals the diversity of immune response to the peanut allergen, Ara h 2. J Allergy Clin Immunol 116:893–899
140. Izuhara K, Saito H (2006) Microarray-based identification of novel biomarkers in asthma. Allergol Int 55:361–367
141. Noh G, Ahn HS, Cho NY, Lee S, Oh JW (2007) The clinical significance of food specific IgE/IgG4 in food specific atopic dermatitis. Pediatr Allergy Immunol 18:63–70

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Allergy Frontiers:Diagnosis and Health Economics
Pawankar, R.; Holgate, S.T.; Rosenwasser, L.J. (Eds.)
2009, XXII, 554 p. 9 illus., Hardcover
ISBN: 978-4-431-98293-7