

Diagnosis of Mold Allergy

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Prerequisite to the correct diagnosis and successful management of any allergic condition is the identification of the responsible etiologic allergenic agents.

Fadal, 1981

DEFINITION OF ALLERGY

Allergic reactions have been described for centuries, but they have not been systematically recognized as being allergic entities. One of the first cause-and-effect demonstrations of allergic disease was performed in 1873 by Blackley (1) who showed "hay fever" to be caused by exposure to grass pollen. The term "allergy" was proposed by von Pirquet in 1906 for the designation of an altered reactivity in consequence of the introduction of a foreign substance. The pathophysiological role of the immune system in the definition of allergy was introduced by Farr and Spector (2). Three criteria would be needed for a definite diagnosis of an allergic state:

1. Identification of the allergen;
2. Establishment of a relationship between exposure and the occurrence of symptoms; and
3. Identification of the immunological reactions involved in the disease (3).

The discovery of IgE by Ishizaka (4) and Johansson (5) in 1967 has provided the immunological basis for the understanding of allergy. It should be borne in mind that the common term "allergic diseases" represents disorders involving release of mediators from activated mast cells and basophils, triggered by a variety of agents.

The allergic diseases may be subdivided into (1) atopic (extrinsic), i.e., an IgE-mediated mechanism for release of mediators from mast cells and basophils, and (2) non-atopic (intrinsic), i.e., a non-IgE-mediated mechanism for release of mediators, resulting in disorders indistinguishable from IgE-mediated disorders. In the present review, the term "allergy" is strictly used to denote an IgE-mediated reaction caused by cell-bound IgE on mast cells and/or basophil granulocytes reacting with allergen, which results in release of allergic mediators producing the characteristic symptoms of atopic disease (6).

IDENTIFICATION OF MOLDS

The identification of mold aeroallergens is very problematic. Currently, about 80,000 species of fungi have been described (7). Identification of airborne molds is a prerequisite to evaluating the clinical relevance of the pathogenicity of molds in allergic diseases. Surveys from all parts of the world have shown that spores from a multitude of fungi are ubiquitous in the air we breathe (8). Different sampling techniques result in varying amounts of molds (9). The number of spores might be underestimated, owing to failing growth in culture media, and even with microscopic identification, the actual allergen load might be higher as a result of antigens occurring in particles smaller than spores (8). The use of an immunochemical aeroallergen-assay technique (10) probably represents the best way of measuring allergen exposure, but it has certain limitations in terms of crossreacting allergens. However, as IgE molecules in allergic patients react with allergenic epitopes rather than with whole spores, the immunochemical assays probably have their most evident relevance in estimating the relation between allergen exposure and clinical disease. In daily practice, registration of spores from different mold species is sufficient, as the most common molds causing allergic disease show either no or minimal crossreaction, i.e., share common allergens. Furthermore, the exact clinical relevance of mold spores in individuals can be elucidated only by measuring the allergen load at the nostrils of the patients, as great variations are caused by local meteorological conditions and biotic factors, such as human activity (11,12). The registration of airborne fungi is of fundamental importance for the allergologist working with patients. However, any of the above sampling techniques only gives information about spore exposure, which may be of no clinical relevance if

patients are not sensitized, i.e., have the capacity to respond with an IgE-antibody production. Unfortunately, some of the most common mold spores found in the air, such as *Cladosporium* and *Alternaria*, also clinically represent the causative allergenic agents for most patients sensitive to molds.

MOLD ALLERGY, QUANTITATIVELY AND QUALITATIVELY

Frequency of Mold Allergy

Owing to the widespread occurrence of airborne mold spores, IgE sensitization to mold allergens could be suspected to be a common cause of allergy in humans. Allergic disease related to exposure to environments rich in molds was first described in 1726 by Sir Floyer as severe asthma in a patient who had just visited a wine cellar. Blackley in 1873 (1) produced "bronchial cattarrh" by inhalation of spores from the mold species *Chaetomium* and *Penicillium*. The exact role of mold spores in producing allergic diseases is still largely unknown, which is mainly a consequence of insufficient selection, characterization, purification, and standardization of allergens from a wide range of fungal species (13). Pennington (14) observed positive skin reactions to one or more of 52 mold extracts in 85% of 526 patients. In a study from Sweden (15), 1250 patients were skin-tested with extracts from *Cladosporium*, *Penicillium*, *Mucor*, and *Botrytis*, representing the most prevalent atmospheric mold spores. Of the patients, 10% showed reaction to one or more of the four mold species. Hyde et al. (16) reported that allergy to molds probably accounted for 5% of all cases of asthma and played a subsidiary role in more. A considerably higher incidence of mold sensitization has been observed in the United States with figures ranging from 20 to 29% (17). By gel immunodiffusion and counterimmune electrophoresis, Mazar et al. (18) found antibodies to fungal antigens in about 40% of asthmatics. It is worth noting that the authors measured antifungal IgG antibodies and not IgE antibodies, and that in the same patients, skin sensitivity to molds estimated by prick and/or scratch test was 23% (*Cladosporium*), 18% (*Alternaria*), 12% (*Aspergillus*), and 9% (*Penicillium*). The pathogenetic role of non-IgE fungal antibodies in the investigated patients has not been elucidated, but as the authors could not relate the antibody response to disease, it is probably a cophenomenon of no importance.

In surveys of unselected asthmatics, an even higher frequency is seen. Aas and colleagues (19) performed a skin-prick test and radioallergosorbent test (RAST) in 80 children referred with asthma/rhinitis and a clinical history suggestive of allergy to molds. Depending on the quality of the skin-test extract, positive reactions were elicited in 10–35% using *Alternaria*, 10–43% *Aspergillus*, 13–65% *Cladosporium*, 1–28% *Mucor*, and 3–33% *Penicillium*. The corresponding figures for a positive RAST were 59, 63, 86, 40, and 56%, respectively. In a multicenter study involving 68 children and 62 adults, Malling et al. (20) confirmed these data. Of the patients screened, 80% had a positive skin prick test (SPT) (>+3 reaction) to at least one of the five mold extracts (*Cladosporium*, *Penicillium*, *Aspergillus*, *Penicillium*, *Mucor*). This study clearly documented the importance of the quality of allergen extracts, as the frequency of positive skin reactions to *Cladosporium* increased from about 10% with a commercially available extract to approx 60% with a moderately potent, well-characterized, and biologically standardized extract (Fig. 1). This extract showed not only a higher potency compared with other commercial extracts, but also a broader spectrum of *Cladosporium* allergens. The study showed SPT to have a higher diagnostic capacity than RAST with optimal skin-test extracts, and once more emphasized the crucial role of the extract when evaluating sensitization in an allergic population. The study also showed a high degree of coallergy to other molds (primarily *Alternaria* and *Mucor*) in patients with SPT reactivity to *Cladosporium*. The association seems to reflect a general tendency (genetically determined?) to produce IgE antibodies against mold allergens. Crossreactivity, in the sense of partial identity between epitopes of the tested mold species, has not been found (13).

RELATION BETWEEN SPORE COUNTS AND ALLERGIC DISEASE

For more than 60 years molds have been generally considered to be important causes of asthma, but mold allergy has been the most difficult aspect of aeroallergy to investigate (21).

The problems involved in elucidating the role of mold spores in allergy are related to both absence of detailed information about allergen exposure and lack of standardized allergen extracts, both of which are prerequisites to ensuring that mold allergens do in fact

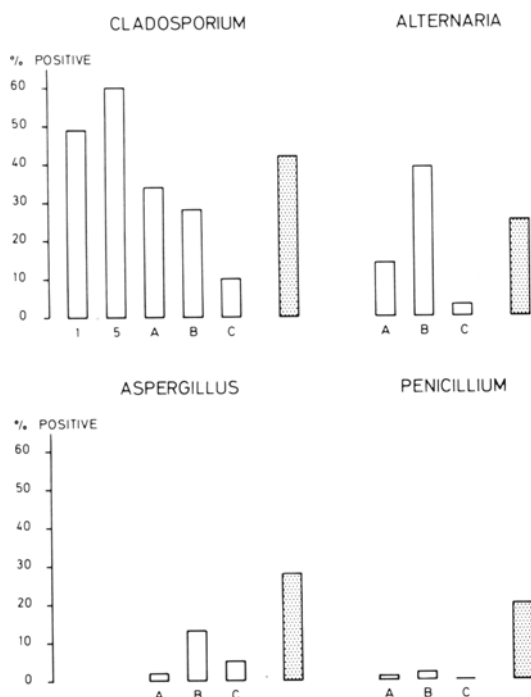


Fig. 1. Screening for mold allergy with four mold species. The percentage of unambiguous positive reactions, i.e., SPT > +3 (open columns) and RAST > class 2 (dotted columns) is plotted for different mold preparations. Columns 1 and 5 represent Pharmalgen® *Cladosporium* 1000 and 5000 BU/mL, and columns A, B, and C are commercial extracts. Reprinted with permission from ref. 20, Malling, H-J. et al., *Allergy* ©1985, Munksgaard Publishers Ltd., Copenhagen, Denmark.

induce disease in sensitized patients. A relation between “asthma epidemics” and climatic changes has been noted (22). The best-documented asthma epidemic associated with changes in weather conditions is the well-known “New Orleans asthma” (23). Based on indirect evidence, Salvaggio and coworkers postulate that the late-summer asthma outbreaks were caused by high levels of atmospheric allergens, especially Ambrosia pollen and spores from deuteromycete, ascomycete, and basidiospores. In a study of 526 mold-extract skin-test-sensitive patients, 13% gave clinical histories strongly suggestive of mold sensitivity (24). Buisseret (25) considered allergy to a given mold spore to be established if the symptoms occurred when the allergen was at its maximal aerial concentration and if by a skin-prick test the patient showed a wheal/flare response to the allergen >5 mm in

diameter. In a survey of 2916 patients, 279 (10%) were allergic to allergens other than grass, 56% were allergic to the spores of *Alternaria*, and 7, 3, and 3% to spores from *Phoma*, *Botrytis*, and *Cladosporium*, respectively (25). In a study of ragweed pollen in autumnal asthma (26), 83% of 29 patients showed a significant correlation between mold spore counts and asthma or hay-fever symptoms, in contrast to 45% who showed a correlation between clinical symptoms and ragweed counts. This is a very interesting observation, as the patients were selected for ragweed immunotherapy based on a history of asthma in the ragweed season, positive skin test, histamine release, and bronchial inhalation challenge with ragweed. Unfortunately, the results of skin tests with mold extracts are not stated.

The first cause-and-effect relationship of molds to clinical allergy in a larger group of patients was published in 1986 by Malling (27). In a study aiming at selecting patients for *Cladosporium* immunotherapy, 22 adult asthmatic patients with well-documented sensitization to *Cladosporium herbarum*, based on positive SPT, RAST, histamine release, and bronchial challenge, recorded daily symptoms, drug consumption, and peak flow for 11 wk during the peak *Cladosporium* season. Although the patients showed skin sensitivity to other inhalant allergens, molds were considered the most important allergens, as patients were either symptom-free (the majority) or had only minimal asthma outside the *Cladosporium* season (late summer and autumn). Patients recorded asthma symptoms twice a day and graded them from 0 (no symptoms) to 3 (severe symptoms), performed peak flow in the morning and the evening, and recorded daily intake of antiasthmatic drugs. Medication was kept at a minimum to avoid complete absence of symptoms. Based on these observations, the relative clinical severity of the asthma disease was related to the *Cladosporium* spore count. The relative severity was obtained by calculating the asthma symptoms continuously in percentages from no symptoms (score 0) to maximum observed (score 100) in the whole recording period. Peak flow and drug consumption were approached in the same way. Correlations were calculated for asthma symptoms (symptom and peak-flow recordings), medication, and the combined score. The mean weekly spore count was correlated to the mean weekly symptom, medication, and total asthma score. A significant ($p < 0.05$) association between concordant increase, decrease, and unchanged values in spore count and symptom, medication, and total score was found

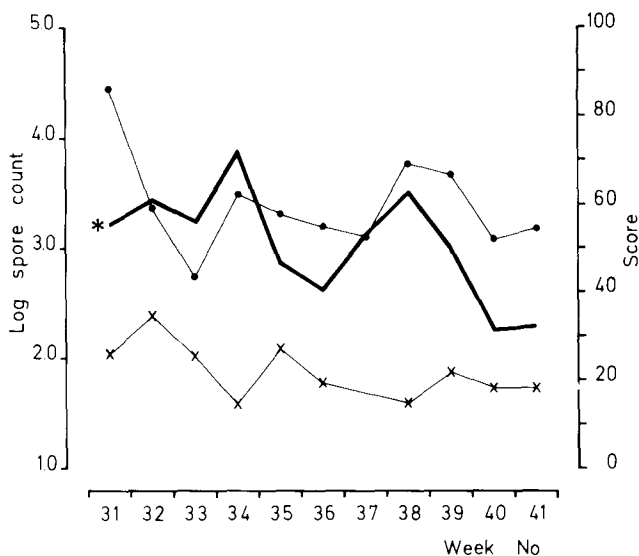


Fig. 2. Relation between weekly spore counts and symptoms, medications and total (combined) scores. | *Cladosporium*, × *Alternaria*; * represents data based on only eight patients. Reprinted with permission from ref. 27, Malling, H.-J., *Allergy* ©1986, Munksgaard Publishers Ltd., Copenhagen, Denmark.

(Fig. 2). Interestingly, an almost inverse relation between *Alternaria* spore count and disease severity was observed, in spite of the fact that 75% of the patients were sensitized to *Alternaria* based on positive SPT and bronchial challenge. The lack of correlation with the *Alternaria* spore count may be attributable to *Alternaria* spores being considerably larger than *Cladosporium* spores, and, therefore, possibly unable to penetrate to the lower airways, hence not producing asthma, but hay fever. By the use of relative scores (change in scores) in contrast to absolute scores (stated using a fixed score system for all patients) in the evaluation between spore counts and clinical symptoms, the problem of interindividual scoring of asthma symptoms and the use of antiallergic medication was avoided.

DIAGNOSTIC PROCEDURES

When discussing diagnostic procedures, it is of fundamental importance to understand some aspects of the relation among IgE sensitization, diagnostic tests, and clinical disease. Traditionally, the diagnosis of aeroallergy is based on a combination of history and

Table 1
Diagnosis of Respiratory Allergy

Test	Biological event
History	Demonstrate "allergic" symptoms in relation to possible allergens and severity of disease.
Skin test	Demonstrate biological activity of the allergen extract caused by mediator release (IgE- or non-IgE-mediated).
RAST	Demonstrate quantitatively the presence of specific IgE, but not the effector pathway.
Challenge test	Demonstrate the capacity of the allergen to elicit a relevant biological response in the shock organ.

in vivo and in vitro diagnostic procedures. It is, however, important to realize that *per se* the various tests do not diagnose only IgE-mediated diseases (Table 1). The skin test demonstrates the biological activity of the allergen in the skin caused by mediator release—but not the IgE antibodies. By the use of purified high-quality allergen extracts, the possibility of a non-IgE-mediated reaction caused by irritants or toxins is negligible. The RAST test quantitatively demonstrates the presence of specific IgE, but not the effector pathway. The in vitro RAST procedure, however, is unique in demanding the detection of IgE antibodies for a positive response, and it is the only reliable test capable of a precise etiological diagnosis based on the presence of IgE antibodies. In other words, RAST represents an immunochemical test system in contrast to, e.g., skin and challenge tests, which represent a complex biological test system dependent on IgE sensitization, mast-cell releasability, and biological responsiveness to mediators of target cells. The challenge test demonstrates the capacity of the allergen extract to elicit a relevant biological response in the shock organ.

In the evaluation of the diagnostic value of a test, the relation between IgE and allergic disease is important. Healthy individuals may be subdivided into "true" healthy and nonsymptomatic atopics, i.e., IgE-sensitized but without clinical atopic disease. In the same manner the atopic population may be subdivided into the nonsymptomatic atopics and patients with a manifest clinical

allergy. When discussing sensitivity and specificity of diagnostic tests, it is important to distinguish between these groups. With an allergological modification, the term "sensitivity" may be defined as the capacity of a diagnostic test to obtain a positive result in IgE-sensitized individuals (nonsymptomatic atopsics and clinically manifest allergics), i.e., a negative test result implies a nonatopic healthy individual. The term "specificity" implies the capacity of a diagnostic test to obtain a negative result in nonsymptomatic individuals (healthy individuals and nonsymptomatic atopsics), i.e., a positive test result implies manifest clinical allergy. In other words, the sensitivity of a test is related to the capacity to detect minimal amounts of IgE, whereas the specificity exclusively represents the capacity to detect IgE in amounts resulting in clinical disease. Owing to the existence of nonsymptomatic atopsics with, indeed, very high levels of specific IgE, the maximal upper limit in terms of specificity for any test will be substantially below 100%, depending on the selection of test subjects. It should be noted that no single test has an inborne maximal capacity with regard to both of these aspects. In the case of both laboratory and challenge tests, sensitivity and specificity may be artificially manipulated by varying the cutoff point between positive and negative test results.

The aim of diagnostic procedures in allergy is to demonstrate IgE-mediated release of mediators in order to establish a specific diagnosis. The methods imply the demonstration of the capacity of the allergen to release mediators and the presence of specific IgE-antibodies—with limitations, however, as the specific diagnosis will always be based on rendering probable a causal connection between the capacity of the allergen to release mediators and the pathogenetic role of specific IgE.

In the opinion of the author, the specific diagnosis represents the combination of demonstrated IgE reactivity (using diagnostic tests) and clinical symptoms related to exposure to the causative allergen (estimated by symptom–medication scores).

History

Fungal spores are always present in the air; rain and snow wash down most, if not all, spores from the atmosphere, and sunshine and wind increase the atmospheric distribution of such spores. The number of airborne mold spores is lowest during the winter months

and highest during the spring and summer months (8), so mold spores, like pollens, in fact have some sort of season, although mold allergy may cause perennial symptoms, often with exacerbations during the midsummer and autumn months (13). Molds require moisture and will not grow unless the humidity consistently exceeds 70% (21). In addition to fungi growing outdoors, molds growing indoors have been suspected as aeroallergens. Studies using simultaneous outdoor and indoor air sampling have shown that the majority of spores have blown in from outside (21). Spores from molds growing indoors are not abundant, and house plants do not bring heavy amounts of mold allergens into the indoor air (28). In bathrooms and damp basements and with poorly maintained humidifiers, buildings may become sufficiently contaminated with mold spores to cause asthma.

In all allergological evaluation the history is extremely important. A thorough knowledge of allergen exposure and the like forms the basis of all diagnostic procedures and represents one of the main arguments for having specialists in the field of allergology. In order to establish a specific diagnosis, the demonstration of IgE sensitization by diagnostic tests does not indicate a clinical role of the IgE antibodies. The history represents the specialist's intelligent way of demonstrating the relationship between exposure and the occurrence of clinical symptoms. In the allergological periodicals, clinical data are usually mentioned only in connection with the evaluation of new laboratory techniques, and stated as positive or negative case history. In the majority of papers the definition of positive or negative history is not described in more detail. Which questions did the patients have to answer? And which algorithm was applied to the answers to decide whether the case history was positive or negative (29)? In surveys of the relative value of the history compared with other diagnostic tests, the history seems almost impossible to use owing to the low predictive value (30). This, however, represents an inverse way of looking at the problem, as the history is the logical gateway to all further testing. The result of the IgE-diagnostic tests always implies a reevaluation of the history in the estimation of the relative clinical importance of the demonstrated IgE sensitization.

The simple information obtained by asking about symptoms during a specified part of the year and relating the answer to pollen counts might be sufficient for a number of patients with symptoms

during a defined season. As asthma is often a multifactorial disease caused by a number of allergic sensitizations, this approach may not be sufficient. Asthma symptoms may be caused not only by the relevant allergen, but also in many patients to an even higher degree by nonspecific factors, such as infections, exercise, air pollution, and the like, resulting in a smouldering clinical picture characterizing allergic disease of the lower respiratory tract. A continuous daily recording of the patient's symptoms (diary cards) has proven to be valuable in the evaluation of asthma (31). Harries et al. (32) correlated spore counts and peak-flow recordings in four asthmatics with positive skin and bronchial-challenge tests to *Didymella*. As stated, falls in peak flow usually coincided with days when spore counts were high, but no direct correlation has been calculated. In the mold-allergy screening study performed by Malling (27), 33 adult asthmatics were selected on a clinical suspicion of mold allergy, as outside the *Cladosporium* season they were either symptom-free (the majority) or had only minimal asthma. Patients completed daily symptom-medication scores in an 11-wk period during the 1981 peak *Cladosporium* season. Patients were defined as having positive allergy (asthma) to *Cladosporium* provided that symptoms oscillated with the *Cladosporium* spore count and there was a positive bronchial provocation test (BPT) at an allergen concentration <10,000 BU (biological units). Negative allergy was defined as no asthma symptoms and a negative BPT. Inconclusive allergy was a denomination for symptoms during the *Cladosporium* season without oscillation with the spore counts or an equivocal BPT. Positive clinical allergy (asthma) was observed in 22 patients and negative clinical allergy in six patients. In only five patients (15%) an inconclusive diagnosis was noted. Concordant increases and decreases of symptom score, medication score, and the combined (total) score for patients with a positive allergy were significantly ($p < 0.05$) associated with changes in *Cladosporium* count.

Skin Testing

Owing to a low potency of mold extracts, the intradermal or intracutaneous test (ICT) has been recommended as the optimal skin test (13,31,33-35). The technical performance and interpretation of the skin response have been the subject of several recently

published guidelines (36,37). Generally speaking, an SPT is recommended as the skin test of choice because of the safety, easy technical performance, and relation to clinical disease. For practical purposes, a negative SPT using potent allergen extracts rules out clinically important allergy. If only low-potency allergen extracts are available, a supplementary ICT should be performed. Optimally, SPT is performed with a specially designed, disposable single-use prick device. The dose and criteria for positive tests with regard to both sensitivity and specificity in relation to clinical disease call for more research. The wheal reaction to the negative control solution may amount to 5 mm² (mean wheal diameter, 2.5 mm) and only reactions 7 mm² or larger should be regarded as positive (37). The risk of eliciting adverse reactions by ICT necessitates some restrictions (38). With the availability of potent aeroallergens, ICT has no clinical advantages over SPT (36,39). In general, ICT should be performed only if the SPT result is negative. The advantage of ICT is the higher sensitivity when testing with low-potency extracts. The clinical relevance of a positive ICT with potent extracts requires further evaluation. The doses and criteria for positive tests with regard to both sensitivity and specificity in relation to clinical disease call for more research (36). In the US, the frequently used criterion for positivity is a + 2 reaction (35), i.e., a wheal of 5–10 mm and an erythema of 21–30 mm. The higher reproducibility of ICT should be balanced by the demand of the test for a very high level of technical skill (36,39).

A systematic comparison between SPT and ICT with mold-allergen extracts has been published by Malling (39). Simultaneously, SPT and ICT in quadruplicate with either *Cladosporium* or *Alternaria* extracts were performed in 25 adult asthmatics with positive SPT, RAST, and BPT. Allergen extracts were lyophilized, and reconstituted and diluted on the day of the testing. The relation between allergen-induced wheal area and reproducibility is shown in Fig. 3. The overall median reproducibility of allergen-induced wheal area in SPT showed a coefficient of variation (SD%) of 29% vs 17% in ICT. The lowest degree of reproducibility (highest SD%) in SPT was found with very small wheal areas (<5 mm²). By linear regression, the slope of the regression line is an expression of the wheal/flare-area increase as a function of the allergen-concentration increase. The median log slope of SPT was 0.32, corresponding

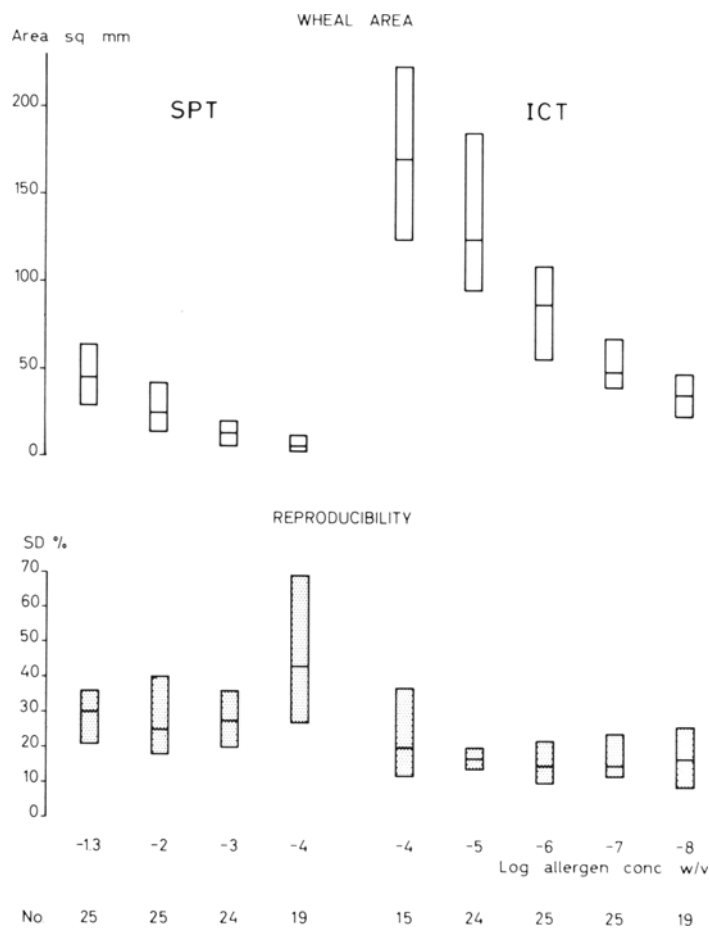


Fig. 3. Relation between allergen-induced wheal area (open columns) and reproducibility (dotted columns) as a function of allergen concentration in SPT and ICT. The number of quadruplicate tests at each concentration is stated. Median and 0.25–0.75 quartile values are marked. Reprinted with permission from ref. 39, Malling, H.-J., *Allergy* ©1985, Munksgaard Publishers Ltd., Copenhagen, Denmark.

to approximately a doubling of the area by a tenfold increase of allergen concentration. The corresponding value for ICT wheal area was 0.22 and for ICT flare, 0.24, equivalent to an increase of the area by a factor 1.7. The coefficient of correlation always exceeded 0.9 in cases of SPT and ICT wheal area, but was significantly lower in cases of ICT flare. We found a significant correlation (Spearman's

$Rho = 0.62$, $p < 0.001$) between ICT and SPT skin sensitivity estimated as the allergen concentration eliciting a reaction of the same size as the histamine control solution (histamine HCl 1 mg/mL in SPT and 0.1 mg/mL in ICT). Skin sensitivity estimated by end-point titration showed no significant correlation between ICT and SPT ($Rho = 0.25$). The results found in this study are highly important when a skin test is used for biological standardization, but have only limited application to routine diagnostic work, in which the key point is to differentiate between a negative and a positive reaction. When skin tests are used in the evaluation of the activity of antiallergic drugs or to monitor allergen-specific immunotherapy, we would recommend quantitative skin-test titration with fourfold determinations of 3–4 tenfold allergen dilutions. To get optimal information from the skin test, an unequivocal dose–response curve (coefficient of correlation > 0.9) and a high reproducibility ($SD\% < 40$) are mandatory. Provided these criteria are fulfilled and potent allergen extracts are available, ICT represents no advantage over SPT.

RAST

The allergy diagnosis based on a radioallergosorbent test (RAST) has limitations resulting from technical conditions. The RAST principle consists of the binding of an allergen extract to a matrix, incubation with patient-specific IgE, and detection of allergen-bound IgE (40). Both the quality (composition and potency) of the allergen extract and the reproducibility of the coupling process in terms of ensuring the binding of all relevant epitopes are of crucial importance for the value of the test (41,42). Specific IgE in patient serum is allowed to bind to the allergen and the number of IgE molecules bound are detected by a radio-labeled or enzyme-marked anti-IgE. The ε -specificity of α -IgE is fundamental for preventing interference from other immunoglobulins. It is worth noting that the Phadebas® RAST is the most well-documented test and should therefore be chosen as the reference test (43). The application of new RAST techniques should be supported by sufficient quality documentation.

The RAST procedure may represent special problems when evaluating mold sensitivity. Coupling of some of the mold allergens to the insoluble matrix may be limited because of the significant

amount of glycoprotein, glycopeptide, and polysaccharide antigens, which fix only sparingly because of the relatively small number of amino groups present. Comparing the diagnostic capacity to skin testing, but not necessarily to clinical disease, RAST have a lower sensitivity (44). Another reason for a discrepancy of the results in skin testing and RAST is the use of different allergen material for the two tests (45). Three different allergen extracts were used for RAST, and the mean percentage of uptake of ^{125} I-anti-IgE was 25.4, 14.1, and 5.5, respectively, in nonimmuno-therapy-treated patients. The difference was caused by a difference in both the qualitative and the quantitative composition of the extracts. By the use of the same extracts for skin test and RAST, the difference in the predictive value for bronchial allergy to *Cladosporium* was about 90% by SPT and approx 80% by RAST, a difference that has only a marginal clinical application (46).

Two studies (19,20) have clearly documented the inadequacy of commercially available mold extracts with regard to skin test and RAST. The Pharmacia Phadebas® RAST showed a significantly higher sensitivity than most available skin test extracts; moreover, a purified preparation of *Cladosporium* increased the number of positive reactions. A commercially improved mold disk showed a significantly improved diagnostic capacity as regards *Alternaria* but not as regards *Cladosporium* (47). The updated Phadebas® RAST technique was compared to the previously used technique and to two commercially available specific IgE test kits (Allercoat RAST, VAST™) with six common mold species (*Penicillium*, *Aspergillus*, *Candida*, *Cladosporium*, *Alternaria*, *Mucor*) (48). The study showed that in BPT-positive patients the new RAST had both a lower frequency of negative tests and higher RAST values compared to the other tests. The paper clearly documents the discrepancy among the diagnostic capacities of various IgE-estimating tests.

Histamine Release

Histamine release (HIST) in the diagnosis of allergy was described by Lichtenstein and Osler (49). In one study HIST showed a better diagnostic capacity with mold allergens than SPT and RAST (31). By the autoanalyzer technique, Reinders et al. (50) showed a

lower sensitivity of HIST compared to SPT and RAST, as approx 20% of positive patients did not release histamine. Nüsslein et al. (47) investigated both the HIST from whole blood and isolated basophil leukocytes and found a 95% overall agreement between HIST and BPT in the *Alternaria*-system vs 73% in *Cladosporium*. In the latter allergen extract, both false positive reactions (24%) and false negative reactions (40%) were observed. The problem of non-responders in the histamine-release technique accounts for only a part of the discrepancy; other problems are connected with the obvious disadvantage of working with living cells very sensitive to outer stimuli, and the like. In comparison with other in vivo techniques, such as SPT and BPT, HIST does not take into account the complexity of a biological test system involving not only the IgE sensitization and mast-cell releasability, but also the influence of IgG antibodies and the responsiveness of target cells to mediators. Another problem with HIST is that the test does not exclusively demonstrate the IgE-mediated release of mediators, but may become positive as a result of non-IgE-mediated reactions. In that way, histamine release, like skin tests and challenge tests, demonstrate only the biological activity of the allergen in releasing mediators, i.e., the effector pathway, but not the IgE antibodies, which are demonstrated only by the RAST technique.

Challenge Tests

Challenge tests may be performed as nasal, conjunctival, and bronchial provocation tests. In studies evaluating the relative value of different diagnostic procedures, challenge tests are often regarded as the final answer or the reference standard in relation to clinical allergy (31,46). It is, however, worth noting that the phenomenon of eliciting bronchial obstruction by BPT in sensitized patients who have never experienced asthma, which is well known from daily clinical practice, calls for some reservations. Furthermore, challenge tests should be performed in the relevant shock organ to prove the pathological role of the allergen, i.e., challenge tests should be performed in the nose when rhinitis is concerned, and in the lungs when asthma is concerned. The technical performance of challenge tests, interpretation, and the influence of pharmacological agents have recently been reviewed by Naclerio et al. (51).

An interesting and clinically relevant inhalation challenge test was performed by Harris (52) by room tests with *Alternaria*. The mold room test was performed by spraying into the room 1 g/700 ft³ *Alternaria* spores and keeping the spores circulating by means of fans. Patients stayed in the room until symptoms developed, or for a maximum period of 1 h. Two groups of patients were tested: 12 patients, all of whom had a typical history of mold allergy and positive ICT to *Alternaria*, and 10 patients in whom the diagnosis of mold allergy could not be made from the history in spite of positive skin test. Eight patients in the former group developed asthma or rhinitis within 10–60 min (67%) and two additional patients developed symptoms within 2–3 h (total, 83%). In the latter group only one patient developed symptoms after exposure for 1 h (10%). This challenge technique approaches the clinically natural exposure, although, like all other challenge procedures, it has the obvious disadvantage of using extremely high quantities of allergen. In a nasal challenge study (24), 22 of 61 patients (36%) developed symptoms from cotton swabs pulled over the surface of various mold colonies. Four of the patients additionally developed asthma. Halpern et al. (53) studied 126 skin-test (scratch and ICT) positive patients with three different preparations of *Alternaria*. The frequency of positive reactions varied from 23 to 63%, equivocal from 0 to 16%, and negative from 37 to 41%. Equally important, in 23 skin-test-negative patients, the challenge test was positive in one patient only. The conclusion of the study was that negative nasal challenge in skin-test-positive patients strongly indicate that the allergen should not be considered responsible for nasal symptoms. The clinical relevance of a positive challenge test was not reported.

Performing bronchial challenge tests with *Cladosporium*, Nilsson and Aas (46) found no positive reactions in 41 controls. In 23 children a full agreement (positive and negative) was found in 96% with regard to skin test (SPT), and in 83% with regard to RAST. The authors claim that the bronchial challenge test is frequently necessary to reach a decisive diagnosis of bronchial allergy in patients, when other IgE-diagnostic tests are equivocal. The relation between the degree of allergen sensitivity estimated by BPT and SPT, RAST, and HIST with the same batch of *Cladosporium* was significant, although not impressive and with wide individual

variations (Spearman's Rho 0.59, 0.52, and 0.48, respectively) (54). The specificity of bronchial challenge in eliciting a positive response only in skin-test- or RAST-positive patients has been shown in a number of studies (54–56). The importance of performing challenge in the relevant shock organ to prove the pathological role of the IgE sensitization is clearly documented by Lopez et al. (56), as only 63 of skin-test- and RAST-positive subjects demonstrated a significant decrease in FEV_1 by bronchial challenge.

A clinically relevant and well-performed study of the role of *Alternaria* and *Penicillium* in the pathogenesis of asthma was published by Licorish et al. (57). Seven adult patients with mild asthma and strongly positive skin tests (SPT) to the investigated allergens were studied. Bronchial challenge tests were performed with both aqueous extracts (delivered by a Wright nebulizer) and intact spores delivered by a Spinhaler. Control experiments were made with diluent and lactose, respectively. The criteria for a positive bronchial challenge were a decrease of FEV_1 of 20% or more, peak flow 25% or SG_{aw} 35%, and clinical evidence of asthma. The provocative doses for both aqueous extracts and intact spores were calculated and compared with environmental spore counts.

The study (57) confirmed that spores from both *Alternaria* and *Penicillium* induced immediate asthma in sensitized patients. The spore challenge with *Alternaria* is of special interest, as the rather large size of the spore ($>5 \mu m \times >15 \mu m$) was expected to prevent it from entering into the lower airways. In half of the patients less spore material from whole spores than from the spore extract was required for a positive challenge, indicating that intact spores may contain potent allergens either not removed by the extraction process or degraded during the processing of the extract. The difference in potency between intact spores and aqueous extract was reinforced by the observation that only whole-spore challenges showed a dual asthmatic response, i.e., both immediate and delayed-type asthma.

Most challenge studies do not mimic the natural exposure of allergens. In ragweed bronchial challenge, quantities of allergen extracts encountered only after an entire season's exposure was required to elicit a positive response (58). The study by Licorish et al. (57) showed that by equating spore-count data with challenge doses, asthma was evoked by cumulative spore doses that would be inhaled over a 24-h period.

RELATION BETWEEN DIAGNOSTIC TESTS AND CLINICAL DISEASE

As stated previously, it is crucially important to differentiate clearly between IgE sensitization estimated by IgE diagnostic tests and clinical manifest allergy, which represents a combination of demonstrated IgE antibodies and clinical symptoms related to natural exposure to the allergen in question. The problem is related to the fact that diagnostic tests only detect IgE in different compartments, but are unable to evaluate the clinical importance of the demonstrated IgE. The use of challenge tests does not solve this problem, as, e.g., bronchial challenge tests may turn out to be positive in asymptomatic subjects. On the other hand, negative challenge tests with relevantly potent and allergenic representative extract rule out clinically important allergen-specific disease in the examined shock organ. Most studies have used, e.g., bronchial challenge tests as an expression of "the true diagnosis" without convincingly documenting the relation between a positive challenge test and clinical symptoms oscillating with the causative airborne allergen concentration. "False positive" IgE diagnostic tests might represent

1. A true "false positive" reaction caused by irritants in the extract (BPT, SPT, HIST) or by nonspecific binding to the allergosorbent (RAST);
2. Compartmentalization or latent allergy, i.e., specific IgE localized in the skin, e.g., but not in other shock organs or circulating; or
3. Positive tests in patients with rhinitis caused by the test allergen, but without asthma, i.e., negative BPT and no asthma symptoms.

The only study trying to correlate the outcome of diagnostic tests to the severity of mold-induced asthma disease was published by Malling (27). Positive allergy = asthma was defined as an unequivocal, positive clinical history of *Cladosporium*-induced asthma combined with a positive bronchial challenge (to ensure the shock-organ reactivity of the allergen extract). The severity of the asthma disease was assessed separately for 24 adult asthmatics by daily-symptom, peak-flow, and drug-consumption recording during 11 wk in the peak *Cladosporium* season. The sum of mean asthma-symptom score, mean peak-flow score, and mean medication score represented the total severity of the asthma disease and was used for calculating the correlation of diagnostic-test sensitivity and the

severity of asthma disease. The sensitivity of BPT was stated as the allergen concentration causing a 20% decrease in FEV₁, skin sensitivity as the allergen concentration eliciting in a titrated skin test a wheal equal to the histamine 1 mg/mL reference, RAST as PRU (Phadebas® RAST units), and HIST as the allergen concentration resulting in a 20% histamine release. No significant correlation of individual mean score and the result of the diagnostic tests was demonstrated. The highest correlation was observed for BPT sensitivity ($\rho = -0.39$) (Fig. 4). The correlation between BPT sensitivity and the individual elements of the total score was $\rho = -0.33$ in relation to medication score, $\rho = -0.30$ in relation to peak-flow score, and $\rho = -0.10$ as regards asthma score. Contrary to hay fever (59), there was no significant correlation between the degree of asthma symptoms and the objective measurements of allergic sensitivity as estimated by BPT, SPT, RAST, and HIST. In spite of the problems of performing and interpreting BPT, this objective test for allergy to *Cladosporium* appears to be the most preferable for predicting the severity of the disease. This indicates the complex nature of asthma and emphasizes that the diagnostic tests are useful only in revealing IgE reactivity, and are of limited value in estimating disease severity.

CONCLUSION

The problems of elucidating the role of mold spores in allergy are related to both an absence of detailed information about allergen exposure, and lack of standardized allergen extracts. Provided qualitatively and quantitatively optimal extracts are available, the IgE-diagnostic tests are of equal value in diagnosing mold sensitization, as with other aeroallergens. In the opinion of the author, the specific diagnosis represents the combination of demonstrated IgE reactivity (using diagnostic tests) and clinical symptoms related to exposure to the causative allergen. In order to diagnose organ-specific disease, an unequivocally positive challenge in the relevant shock organ is essential. The rational approach to diagnosing mold allergy is, based on the clinical history, to use skin test as the primary screening test. SPT has the highest sensitivity (few false negative reactions) and, compared to ICT, few irrelevant positive reactions, and

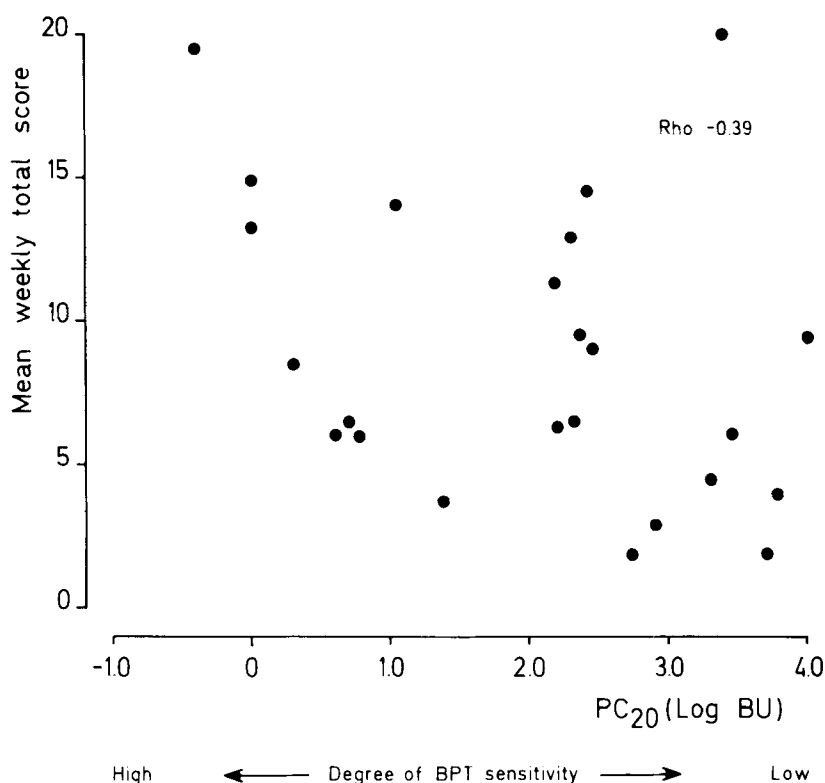
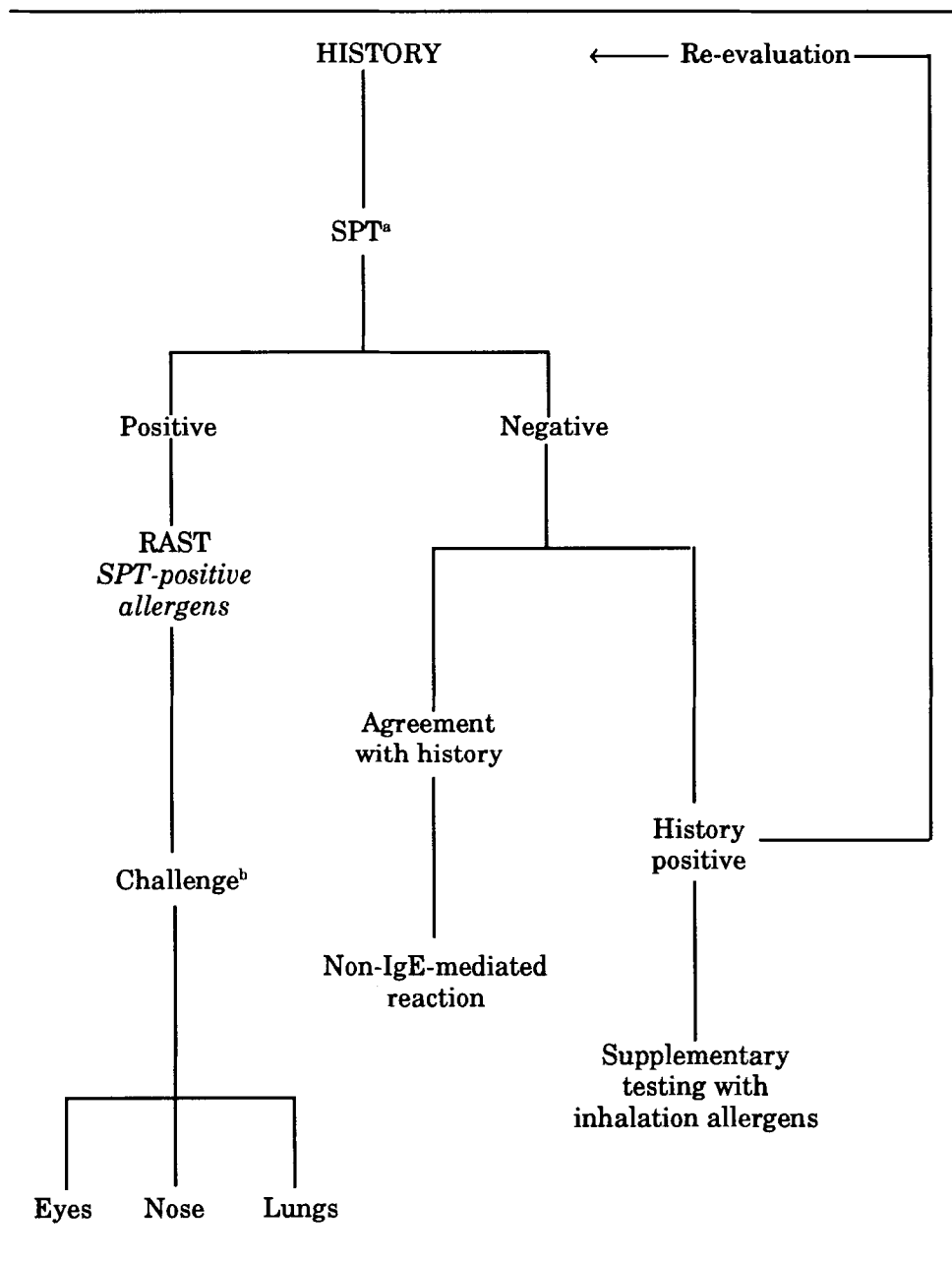


Fig. 4. The correlation between BPT sensitivity (PC_{20}) and mean combined symptom and medication score during an 11-wk observation period in the peak *Cladosporium* season. Reprinted with permission from ref. 27 *Allergy*.

should consequently be used to screen for IgE sensitization in the diagnostic workup. Because of the lower sensitivity, RAST is not optimal as the initial test, but as a result of high specificity (few false positive reactions) it is optimal as a confirmatory test for the presence of specific IgE. The clinical relevance of the IgE sensitization should be confirmed by reevaluating the history to ensure that the patients do in fact have symptoms caused by the allergen (Table 2). Challenge tests are normally indicated only if the diagnosis of allergen sensitization implies therapeutic interventions, such as allergen-specific immunotherapy.

Table 2
Standard Allergy Diagnostic Procedures
(*ad modum Malling*)



^aAvoid SPT in case of eczema and use of antihistamines.

^bIndicated only if the result implies therapeutic interventions.

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