

Phadiatop—a novel IgE antibody screening test

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Summary

The Phadiatop® test, which is based on a multi-allergen allergosorbent, proved to be a test that is simple to perform in the laboratory and produces reliable results. When compared with the more conventional RAST atopy screening test for grass, mite and IgE antibodies it produced similar results, except in those rare instances of patients who were RAST-positive only for moulds where the Phadiatop test was decidedly superior. The Phadiatop test disc contains only inhalant allergens and so it could not be used for screening infants and very young children whose IgE response, if any, is probably limited to foods.

Introduction

Atopic allergy affects approximately one-third of people under 30 years of age who live in industrialized countries. It is also a problem in non-industrialized countries, but its extent is less well documented. The most common diseases associated with atopy are asthma, rhinitis and eczema, but other skin and even gastrointestinal complaints may also be atopic in nature. However, it has to be recognized that these diseases have non-allergic counterparts, i.e. antibodies do not participate in their pathogenesis. Furthermore, in some instances, especially those involving delayed hypersensitivity reactions, while antibodies may be involved they need not be of immunoglobulin E (IgE) class and so, while such cases can be labelled as allergic, they should be clearly dissociated from the commonly employed term of allergic disease, which conventionally refers to IgE-mediated or atopic allergy. To summarize, an atopic person is one who is triggered to produce specific IgE antibodies after inhalation of common environmental allergens [1,2].

It is rare for an allergic attack to prove fatal, but anaphylactic reactions due to drugs, venoms or foods do occur, and recent surveys from all over the world conclude that more people are dying from asthma. However, allergy is usually incapacitating and results in considerable inconvenience due to lost time at work, school and play; in the U.S.A., 35 million people (17% of the population) are affected at a cost per annum of \$US 1.5 billion, and as a result 5 million working days are lost each year [3]. Similar

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figures for probable direct and indirect costs are available from Europe and Japan. The remedy for this situation is not readily at hand because the number of allergies requiring diagnosis and attention is much larger than the numbers of clinicians adequately trained in the subject. Consequently, the introduction of any procedures that can be used by other clinicians as well as allergists to help reduce the burden of recognizing and diagnosing allergy is a worthwhile objective.

With this as the objective, a U.K. survey was conducted to establish which allergens are most likely to trigger an IgE response. Adults and young children were tested for circulating IgE antibodies directed against thirteen inhaled and six ingested sources of allergens; the most commonly identified IgE antibodies were specific for grass pollen, *Dermatophagoides pteronyssinus* and cat epithelium extracts [4]. Furthermore, when the results were subjected to cluster analysis it was found that at least one of these three allergenic sources was a constituent of all but one of the nine clusters—the exception being a small but statistically highly significant cluster of patients who only produced IgE antibodies to moulds. The conclusion made was that a single screening test for grass-, mite- and cat-specific IgE antibodies could identify 94% of the atopics in the U.K. (excluding infants, in whom inhalant-specific IgE antibodies are only rarely identified). A mixed allergosorbent comprising extracts of grass pollen, *D. pteronyssinus* and cat epithelium chemically linked to microcrystalline cellulose particles was shown to be a satisfactory single test for the identification of atopics: the so-called RAST atopy screen [5]. However, because atopy is only an indication of sensitization and, therefore, need not be associated with current symptoms, the clinical usefulness of the atopy screening test was improved when combined with a clinical history scored with the aid of an 'expert system' computer program [4]. Furthermore, the extent to which further tests might be required could be judged by reference to the result of a total serum IgE measurement—the lower the total IgE level the fewer the number of specific IgE tests that should be required. Thus, a combination of the RAST atopy screening test, a clinical history score and a total IgE determination constitutes our original allergy screening system.

The RAST atopy test is the key component of the screening system and can either be employed as a single test utilizing a mixture of particle allergosorbents, which have been quality controlled as to composition and strength before mixing, or as three separate tests using grass, mite and cat paper-disc allergosorbents. Production of the mixed-particle allergosorbent in the first option is quite technically demanding, while use of three separate paper discs in the second deviates from the objective of using a single test for atopy. Obviously, if the test could be made simpler, and perhaps include an increased variety of allergens (inclusion of moulds in the RAST atopy test panel would have increased the atopic catch rate from 94 to 96.5% [4]), then it should be more universally acceptable. We report the results of our satisfactory experience in replacing in our screening system the RAST atopy test by a commercially available paper-disc allergosorbent prepared with a variety of highly purified allergens from pollens, house dust mite, animal epithelia and moulds.

Subjects and methods

Patient material

This was selected retrospectively to test rigorously the performance of the new multi-allergen test called Phadiatop® (Pharmacia Diagnostics, Milton Keynes, U.K.) as a

replacement for the grass/mite/cat-RAST used in our screening system. The evaluation was divided into two stages. In the first stage, 187 sera were grouped according to their assignment as positives or negatives in the three screening tests.

(i) *Total serum IgE*—where a positive patient was regarded as one whose value was greater than, or equal to, 40 kU/litre.

(ii) *RAST atopy test* to detect IgE antibodies directed against Timothy grass pollen, *D. pteronyssinus* and cat epithelium—a positive test was a class '1' or greater RAST score to at least one of these three allergens. The terms RAST atopy test and grass/mite/cat-RAST are used synonymously in the text, and represent the combination of results from the RAST analyses for three individual allergens.

(iii) *Clinical history test*—the history was assessed with the aid of a microcomputer programmed with a set of rules and Bayes theorem of probabilities to interpret answers given by the patient to a detailed questionnaire [6]. A probability of at least 50% or more was termed a positive history.

Thus, eight groups were formed according to the combination of results for these three tests. For example, one group comprised twenty-four patients who were negative to all three tests, three groups had only one test positive, another three groups had two of the three tests positive and the final group had positive results to all three tests. The only group that did not contain twenty-four patients had nineteen positive only to the RAST atopy test. This selection criteria is summarized in Fig. 1. Blood for IgE determinations was sampled throughout the year: two patients from each of the eight groups every month, i.e. sixteen patients per month, excepting the small group of nineteen. It was more difficult to find patients in this category (i.e. with IgE antibodies to at least one of these three common allergens, but with a negative history and total IgE test) than in any of the other seven groups, but we attached no significance to the fact that no patients were found with only the RAST atopy test positive for April and only one patient for each of the months of January, March and August.

The second stage involved testing an unusual cluster of twenty-five patients with Phadiatop. These patients had been screened with RAST against the thirteen inhalant allergens that comprised our allergen screening panel and were positive to only one or more of the moulds. Clinical histories were only available from some of these patients, but our purpose was to discover if Phadiatop could 'catch' this admittedly small, but statistically highly significant group of patients, which would not be 'caught' by the RAST atopy test.

Immunoglobulin E tests

(a) *Total serum IgE*. Measurements were performed using Phadebas-IgE PRIST kits (Pharmacia Diagnostics Ltd, U.K.) according to the manufacturer's instructions, except that all sera were assayed at five- and fifty-fold dilutions in duplicate. The test procedure was controlled using high (340 kU/litre), medium (180 kU/litre) and normal (70 kU/litre) control sera and the between-assay coefficients of variation were 6.9, 4.5 and 6.0%, respectively.

(b) *RAST*. Circulating levels of IgE antibodies specific for Timothy grass, mugwort, plantain, birch tree and plane tree pollens, *D. pteronyssinus*, cat epithelium, dog and horse danders, *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus* and *Alternaria tenuis* were estimated using Phadebas IgE reagents (Pharmacia Diagnostics Ltd, U.K.). The procedure [7] was a modification of the manufacturer's using test and reference sera diluted three-fold to a final volume of 150 μ l. The first

incubation of serum with paper-disc allergosorbent was overnight, and after a washing step any allergen-IgE complexes were detected using ^{125}I -anti-IgE in a second overnight incubation step. This procedure is as sensitive as the one recommended by the manufacturer but limits the falsely low classification of IgE antibody levels that can exceptionally occur when sera contain high levels of the IgE-antibody being tested. It was quality controlled with RAST class '2' positive sera for each allergosorbent.

(c) *Phadiatop IgE antibody screening test.* This two-step sandwich radioimmunoassay employed a paper-disc multi-allergosorbent to which mixture of grass, mite, cat and other common U.K. inhalant allergens was attached. The precise formulation was not disclosed but included a variety of pollens, mites, moulds and animal danders. The first step involved horizontally shaking 50- μl of test or control serum with the allergosorbent for 1 hr at room temperature and, after thoroughly washing the disc allergosorbent, the second step required it to be shaken for a further 2 hr with ^{125}I -anti-IgE (50 000 c.p.m.). All results were compared to those obtained using a reference serum and an anti-IgE reference disc in an identical procedure, and reported as positive if the radioactivity associated with the test serum exceeded that for the reference serum. The assay was controlled by interspersing the control serum with every tenth test serum.

Results

Comparison of screening tests for atopy: Phadiatop vs grass/mite/cat-RAST

The two tests gave concordant results in 179 of the 187 patients (95.7%) studied in detail (Fig. 1). For the ninety-six sera that had no detectable IgE antibodies to grass, mite or cat allergosorbents, ninety-three (96.9%) produced negative Phadiatop results, whereas from among the ninety-one grass/mite/cat-positive sera, eighty-six (94.5%) were Phadiatop positive.

The result of substituting Phadiatop for the grass/mite/cat-RAST is summarized by the Venn diagram in Fig. 2. The pattern observed is similar to Fig. 1 but for a closer inspection of the discordant results it is necessary to compare the tests according to the eight patient groups.

Selection of sera

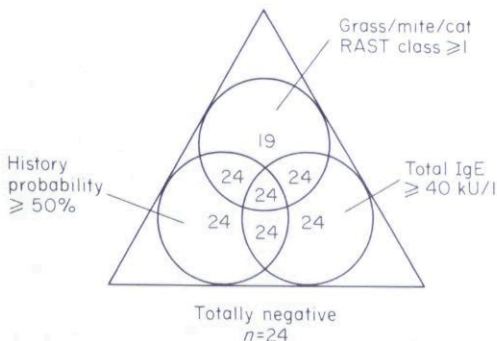


Fig. 1. A Venn diagram that describes how the patient material was placed into eight groups according to their assignment as negatives or positives in screening tests of total serum IgE, clinical history atopy score and grass/mite/cat-RAST atopy screen.

Results of screening tests

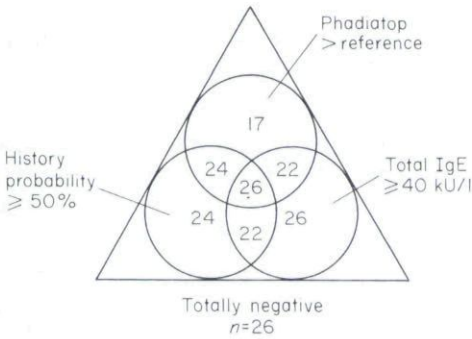


Fig. 2. A Venn diagram that summarizes the experience of substituting the grass/mite/cat-RAST screen with Phadiatop.

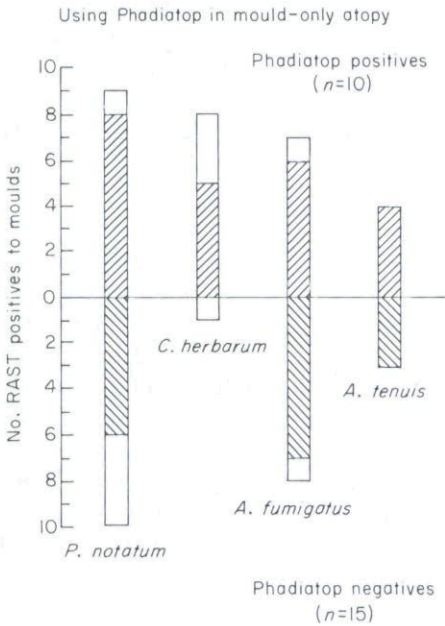


Fig. 3. Twenty-five sera tested by Phadiatop and by RAST for IgE-antibodies to four moulds: *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus* and *Alternaria tenuis*. (■) Number of sera with mould RAST scores greater than 1; (□) patients with scores of 1. Results are divided according to whether Phadiatop results were positive (ten patients above the zero line) or negative (fifteen patients below the zero line). Only in the case of *Cladosporium* were positive RAST results clearly associated with positive Phadiatop.

(a) *Groups I and II—both history and total IgE tests negative.* Phadiatop was negative for all the patient sera that were grass/mite/cat-RAST negative, but of the nineteen who were grass/mite/cat-RAST positive, two were Phadiatop negative.

(b) *Groups III and IV—history positive and total IgE negative.* Of the twenty-four sera that were grass/mite/cat-RAST negative, only one was Phadiatop positive; similarly, in the other group of twenty-four grass/mite/cat-RAST positive sera there was one serum that was Phadiatop negative.

(c) *Groups V and VI—history negative and total IgE positive.* All twenty-four sera that were grass/mite/cat-RAST negative were also Phadiatop negative, but two of the twenty-four sera that were grass/mite/cat-positive were Phadiatop negative.

(d) *Groups VII and VIII—history positive and total IgE positive.* Phadiatop was positive for all the twenty-four sera that were grass/mite/cat-RAST positive, but was also positive for two of the fourteen that were grass/mite/cat-negative.

Therefore, overall there were eight discordant results and in seven instances it was the Phadiatop result, and not the grass/mite/cat-RAST result, that supported the clinical histories. Consequently, this small apparent loss in sensitivity may be clinically unimportant.

Mould cluster group

These twenty-five sera were selected because they were grass/mite/cat-RAST negative, but positive to at least one of the four moulds included in the RAST panel. The results are summarized in Fig. 3, ten of the twenty-five sera (40.0%) being Phadiatop positive. It is apparent that of the nine patients who were *C. herbarum* positive, all but one were Phadiatop positive, but the correlations between RAST and Phadiatop results were not so good for the other moulds.

Discussion

It is estimated that about 30% of the populations in 'industrialized' countries suffer from symptoms associated with atopic allergy. Furthermore, the incidence appears to be increasing [8,9], although whether this is a world-wide phenomenon or merely due to better diagnoses, is not entirely clear [9]. Certainly, IgE tests have been powerful research tools for studying the mechanisms of atopic allergy but, because allergy is such a large clinical problem, it is safe to predict that routine IgE tests will become faster and simpler to perform whilst being more efficient and reliable in providing diagnoses. Such tests should be able to assist practitioners in deciding whether IgE-mediated allergy is likely to be contributing to the symptoms presented, and if the patient should be treated or referred to an allergy clinic. However, allergists and consultants in adjoining specialities often have similar needs to the practitioner. For example, dermatologists need to know whether a contact allergy is the problem—if the patient is atopic the search for a contact agent may be unnecessary. Similarly, ear, nose and throat consultants must decide whether their patients should undergo surgery or whether conventional allergy treatment and advice is required.

The Phadiatop test aims to provide all clinicians with a 'Yes' or 'No' answer to the question: 'Is this an atopic patient?' Despite a slightly lower sensitivity than the grass/mite/cat-RAST, we have no doubt that the test can be used for this purpose. Strictly speaking we have not selected our patients to substantiate this claim, i.e. the Phadiatop test has not been used to identify what proportion of the U.K. population, aged more than 5 years, is atopic. Rather, ours has been a critical evaluation of the test to see how well it can be substituted for the more usual RAST atopy test for grass, mite and cat IgE antibodies, both using it alone and together with clinical histories and total serum IgE levels in our routine allergy screening system.

Our evaluation suggests that the Phadiatop test is an improvement over the grass/mite/cat-atopy test because it was able to identify, (i) a similar number of atopics than the RAST atopy screen (87/91) and (ii) it succeeded in positively identifying about half (40.0%) of the atopics in the mould cluster group, whom by selection had no IgE antibodies to grass, mite or cat antigens. Consequently, the inclusion of mould allergens is justified, while allowing that there is still some room for improvement. The Phadiatop test should prove much more cost-effective than screening with a large panel of allergens by RAST—a procedure already pointed out to be most unrewarding [10].

Inevitable considerations when attaching a large number of allergens to a paper disc are (i) whether the most appropriate allergens have been selected and attached in sufficient concentrations and (ii) whether an accumulation of IgE antibodies, which although individually would have been classified as negative, would produce a positive Phadiatop result. In order to answer these questions a detailed study of Phadiatop's sensitivity and specificity is under way. However, it is clear that the first consideration has been satisfactorily answered—with the proviso of room for improvement in attaching moulds—and there was certainly no evidence of artefactual false positives if the second consideration was to be a problem. From our results it is evident that Phadiatop is suitable for screening in the U.K. and probably for most of Northern Europe.

In performance the Phadiatop test required about 4 hr to screen 100 sera. This allows for same-day turn-round of specimens, but it is more suited as a test for the laboratory rather than the doctor's office.

The atopic person can produce both positive RAST and skin-prick test results as evidence of sensitization. However, these need not be associated with current symptoms and so it is essential that Phadiatop test results be interpreted together with clinical histories. Consequently, we believe that use of the Phadiatop test should be as a substitute for the grass/mite/cat-RAST in the screening system, rather than as a replacement for both this and the total IgE and history tests. For example, the clinical history may strongly indicate some source of allergen not represented by Phadiatop, which could be a food (especially important in infants and young children), venom, drug or an allergen only encountered in a factory; on the other hand the history could be so complex as to indicate that many different allergens are relevant and a significantly raised total serum IgE would support this indication. The total serum IgE is still a test whose main use is as an aid to determine the scale on which a search for IgE antibodies should be mounted—the more elevated the value the greater is likely to be the variety of IgE antibodies encountered.

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