

Correlation between skin tests, inhalation tests and specific IgE in a study of 120 subjects allergic to house dust and *Dermatophagoïdes pteronyssinus*

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Summary

The radioallergosorbent test was compared with currently accepted allergy diagnostic techniques in seventy-four controls and 120 patients with a clinical history of allergy to house dust and *Dermatophagoïdes pteronyssinus*.

The results obtained with *D. pteronyssinus* allergen extract show a close correlation between the size of the cutaneous reactions and the serum level of allergen-specific IgE. On the other hand, highly positive skin tests to house dust were not always correlated with a positive RAST response for house dust. Similar results were found with inhalation tests. When a purified *D. pteronyssinus* extract was used, a stronger relationship was observed between RAST, cutaneous and inhalation tests. It can be assumed that RAST for house dust is not of value in patients with house dust and mite allergy. Our findings indicate that the correlations between RAST results and other *in vivo* test results depend on the purity of the allergen extracts and the selection of patients.

Introduction

The importance of *Dermatophagoïdes pteronyssinus* (*D. pteronyssinus*) in house dust respiratory allergy has been recognized for a long time. The present study deals with the correlation between results from skin tests, inhalation tests and RAST in 120 subjects with a clinical history of allergy to house dust and sensitive to *D. pteronyssinus*. We have tried to answer the following questions: (a) what are the advantages of RAST in comparison to *in vivo* tests such as skin and provocation tests? (b) Is there a close correlation between results from RAST to house dust and *D. pteronyssinus*? (c) Is the agreement between results from RAST and from the other tests better when purified extracts of allergens are used?

Materials and methods

Subjects tested

A total of 194 subjects was selected: (a) a first control group of thirty-three patients without asthma, (b) a second control group of forty-one patients with intrinsic asthma, Correspondence: Dr G. Pauli, Pavillon Laennec, C.H.U. Strasbourg 67000, France.

but with negative skin test results for house dust and *D. pteronyssinus*, and (c) a group of 120 patients with house dust respiratory allergy who had been referred to the Pulmonary Disease Institute (Pavillon Laennec, C.H.U. Strasbourg, Director: Professor P. Oudet). All these patients had episodes of breathlessness and wheezing, but relative absence of symptoms between attacks.

The patients were said to have clinical features of allergy to house dust and *D. pteronyssinus* if we noted noticeable increase in symptoms on exposure to house dust, diminution of symptoms with altitude and aggravation of symptoms by humidity, especially in the recognized house dust season, i.e. from mid-August to the first winter months, with a maximum in autumn.

Allergens tested

(A) *House dust*. A house dust extract was provided by the Pasteur Institute. All tests were made using the three serial ten-fold dilutions: 1/5000, 1/50,000 and 1/500,000.

(B) *D. pteronyssinus*. (a) A *D. pteronyssinus* extract was provided by the Pasteur Institute. All tests were made using the three serial ten-fold dilutions: 1/10,000, 1/100,000 and 1/1,000,000. (b) A purified extract of *D. pteronyssinus* was provided by the Pasteur Institute and the same serial ten-fold dilutions were used.

The culture of *D. pteronyssinus* was made by the procedure of Araujo-Fontaine (1974). The purified fraction was prepared as follows: the mites having been cultured in the initial medium for several months were then frozen and thawed several times. A 48 hr maceration in borate buffer at pH 8.5 was followed by centrifugation. The supernatant was neutralized and submitted to acetone precipitation at increasing concentrations. The fraction precipitated at 80% acetone was isolated, washed and dried. This purified extract was lyophilized or stocked as a solution for cutaneous tests in the presence of 50% glycerol and 5% phenol.

Methods of testing

Skin tests. Intra-cutaneous injections of 0.04 ml were given with the three concentrations of the test extract. The mean diameter of the weals was measured to the nearest mm 20 min after the test.

Bronchial provocation tests. Provocation tests were made with non-glycerinated extracts from the same batches as those used in the skin tests. The patients were instructed not to take any drug for 24 hr beforehand and had to be symptom-free at the time of testing. The aerosols were produced in a Piglowski bronchotest, with a continuous output of aerosol enabling the patient to inhale a constantly renewed aerosol, and indicating the exact quantity inhaled. The patient's FEV₁ or the airway resistances were measured 15 min after each inhalation of an increasing quantity of allergen. A variation level of 100% for airway resistance and 25% for FEV₁ was taken as positive.

RAST (Phadebas tests). Blood samples were taken prior to other investigations. RAST determinations were made at the Institute of Bacteriology and Immunology in Strasbourg (Director: Professor R. Minck). In Phadebas RAST, the allergen house dust (H2: Hollister-Stier Laboratory) or *D. pteronyssinus* (Bencard Laboratory) were covalently coupled to paper discs and reacted with the specific serum IgE. Results are expressed in RAST scores from 0 to 4: class 0 is considered as negative, class 1 as doubtful, classes 2, 3, 4 are considered as positive with progressively increasing amounts of specific IgE.

Results

Relationship between skin test reactions and RAST results

(a) In a control group of thirty-three patients without asthma: there were two subjects with a positive RAST for *D. pteronyssinus*; no RAST result was positive for house dust. (b) In the forty-one subjects with intrinsic bronchial asthma and negative skin test reactions, the RAST results for house dust and *D. pteronyssinus* were negative. (c) Out of the 120 subjects with positive skin test reactions for house dust and *D. pteronyssinus*, the relationship between positive skin test reactions to house dust and

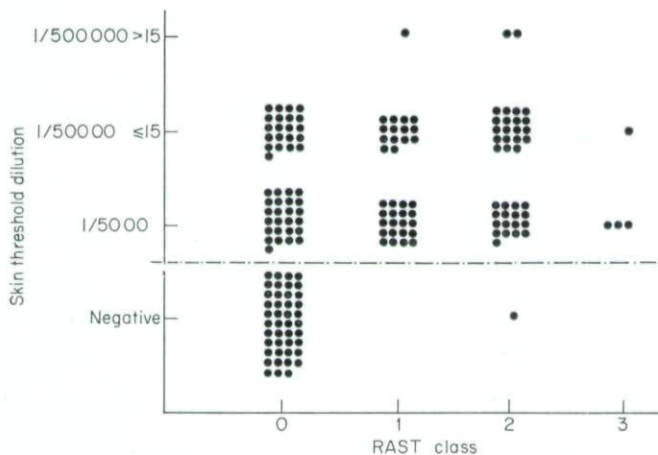


Fig. 1. Comparison between the size of the skin test reaction with serial dilutions of house dust extract and the serum level of house dust-specific IgE ($n = 120$).

RAST was as follows: the RAST response for house dust was positive in seventy-five cases, so that the RAST response for house dust was negative in 38% of the patients. In the same group, RAST results for *D. pteronyssinus* were negative in only 12.5% of the patients.

These results were supported by a more detailed study of the size of the skin test reaction with serial dilutions of allergen extracts and the serum level of allergen-specific IgE. The mean diameter of the weal response to serial dilutions of house dust extracts was measured in 120 patients (Fig. 1).

It was found that highly positive skin tests were not always associated with a positive RAST response for house dust. Out of 120 patients with a positive skin test reaction, forty-six had a negative RAST result, thirty-four a RAST class 1, thirty-six a RAST class 2 and only four had a RAST class 3 for house dust.

A similar study with *D. pteronyssinus* gave different results. The subjects with highly positive skin test reactions to *D. pteronyssinus* (mean diameter of the weal in response to a 1/100,000 dilution of *D. pteronyssinus* extract greater than 15 mm) nearly always had a high level of specific IgE for the same allergen. It is shown on Fig. 2 that a high proportion of these subjects have a RAST class 3 or 4. Yet, it must be noted that in a few subjects having positive skin test results, we were unable to detect specific IgE by the RAST tests.

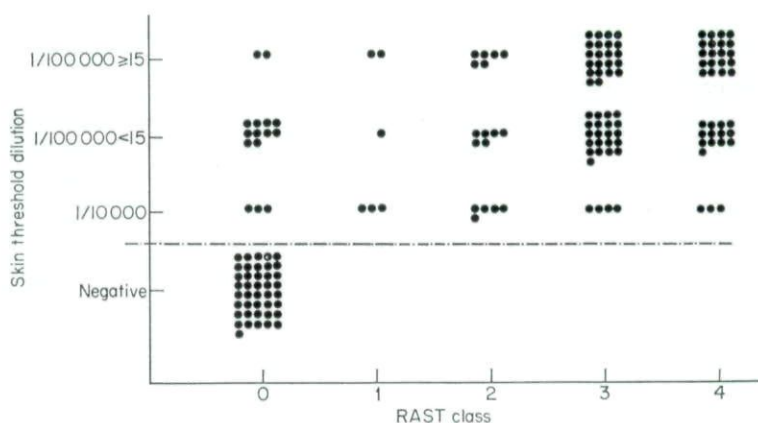


Fig. 2. Comparison between the size of the skin test reaction with serial dilutions of *D. pteronyssinus* extract and the serum level of *D. pteronyssinus*-specific IgE.

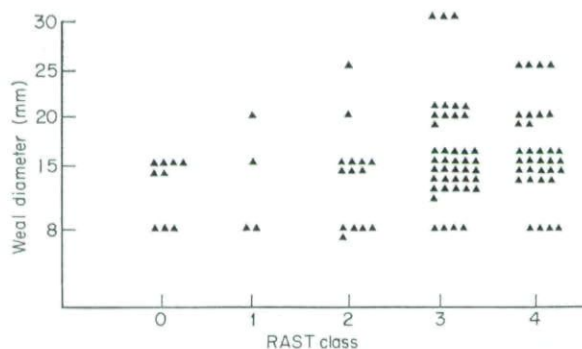


Fig. 3. Comparison between the mean weal diameter of an intradermo-reaction with the 1/100,000 dilution of an experimental purified extract of *D. pteronyssinus* and the RAST classes to *D. pteronyssinus*.

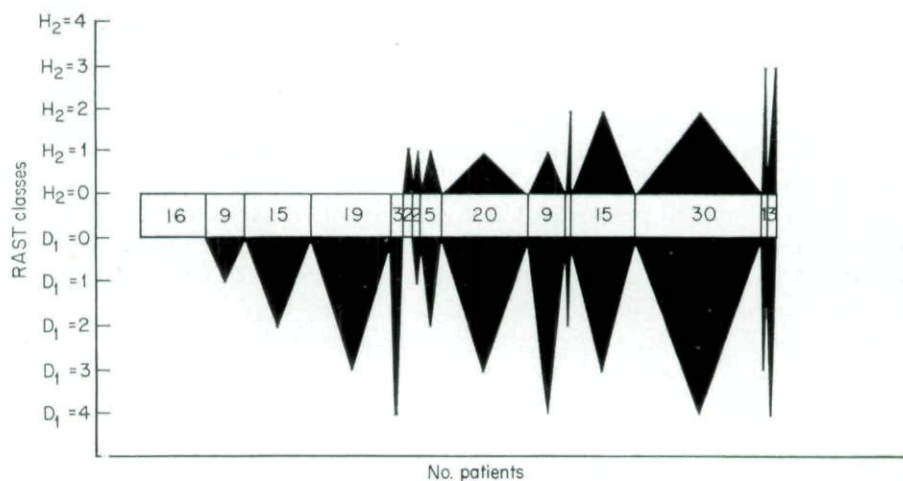


Fig. 4. Comparative study between the different classes of RAST for house dust (H₂) and *D. pteronyssinus* (D₁) on 150 subjects with positive skin tests to house dust and *D. pteronyssinus*.

In 103 subjects, skin tests were performed with an experimental purified extract from the Pasteur Institute. The skin reactions were always positive with a 1/100,000 dilution (Fig. 3). The mean diameter of the weal response to that dilution correlated well with the serum IgE level specific for *D. pteronyssinus*. Our methods of measurements (mean diameter of the weal response and, above all, the classification of the RAST results) are not truly quantitative for statistical analysis.

A comparative study between the different classes of RAST scores for house dust and *D. pteronyssinus* was made in 150 subjects tested with the two allergen extracts: house dust and *D. pteronyssinus* (Fig. 4). Of eighty-one subjects with a highly positive RAST score for *D. pteronyssinus* (class 3 or 4), only 33% had both a positive RAST

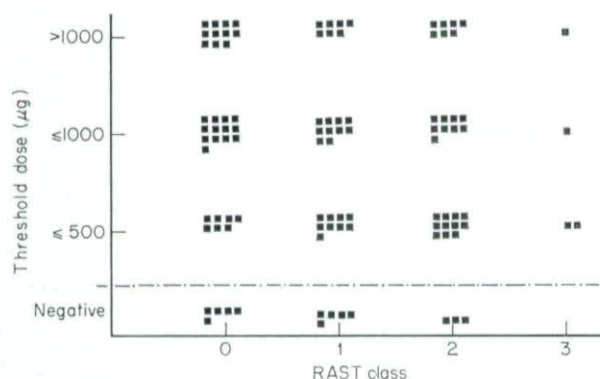


Fig. 5. Comparison between bronchial threshold dose and RAST classes to house dust extract.

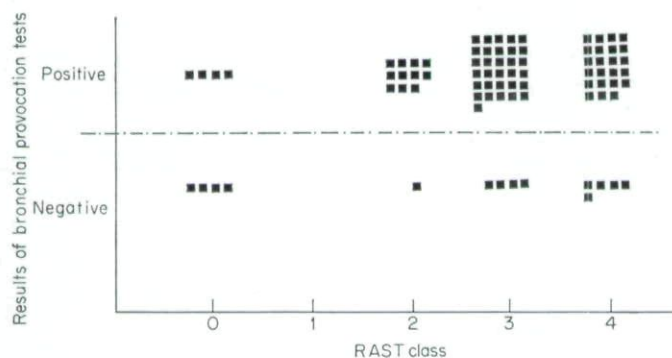


Fig. 6. Values of the serum level of *D. pteronyssinus*-specific IgE compared with positive or negative inhalation tests.

score for it and for house dust. If we consider RAST class 1 for house dust as positive, we obtain a concordance of 54% for the results of the two RAST tests. Negative RAST result for house dust is always associated with negative RAST result for *D. pteronyssinus*. We must emphasize that all of the subjects had highly positive skin test reactions for the two tested allergen extracts, but the extracts used for the skin tests and RAST were provided by two different laboratories.

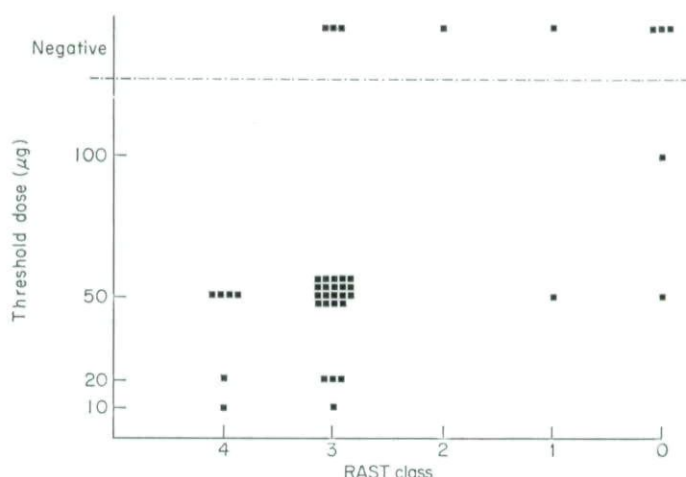


Fig. 7. Comparison between bronchial threshold dose and RAST classes to a purified experimental extract of *D. pteronyssinus*.

Relationship between inhalation tests and RAST results

Bronchial provocation tests were made on 101 patients, using a house dust allergen extract. The bronchial response to this extract was positive in eighty-eight out of 101 subjects. The threshold dose giving a positive inhalation test was compared to the levels of specific IgE (RAST classes) (Fig. 5). For house dust there is a poor agreement between RAST and inhalation test results. Considering RAST class 1 as doubtful, RAST results are highly positive in only 35% of the subjects giving a positive inhalation test result. For this particular allergen, the concordance between inhalation test and RAST results is even weaker than the concordance found between skin test and RAST results.

On the other hand, inhalation tests with the *D. pteronyssinus* extract were made on a group of eighty-three subjects (Fig. 6). Sixty-nine inhalation test results were positive

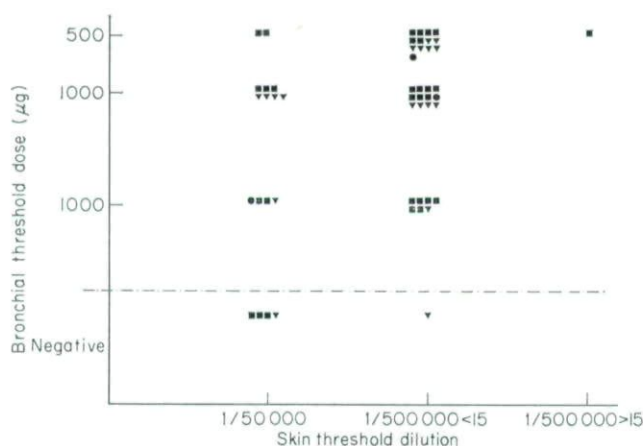


Fig. 8. Comparison between the size of the skin test reactions with serial dilutions of house dust extract, the bronchial threshold dose and RAST classes to house dust. RAST 0 and 1 (■); RAST 2 (▼); RAST 3 and 4 (●).

and in only four of these was the RAST negative. Moreover, in fifty-four of the sixty-nine cases (80%), the class of the RAST was high (class 3 or 4); it appears therefore that there is a more definite correlation between the *D. pteronyssinus* inhalation tests and the RAST results for this than for house dust.

We were later provided with a purer *D. pteronyssinus* extract. Fig. 7 shows that in inhalation tests carried out with this, the RAST results were positive in thirty-two out of thirty-nine cases. It is interesting to note that when bronchial sensitivity is high (threshold dosage $<100 \mu\text{g}$), the level of specific IgE is elevated (class 3 or 4).

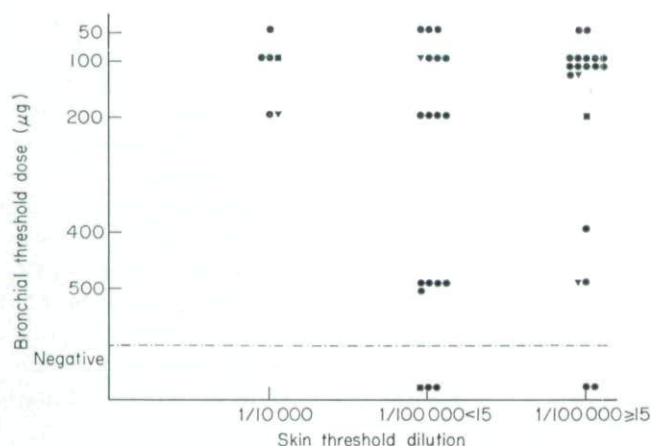


Fig. 9. Comparison between the size of the skin test reactions with serial dilutions of an experimental purified extract of *D. pteronyssinus*, the bronchial threshold dose and RAST classes to *D. pteronyssinus*. RAST 0 and 1 (■); RAST 2 (▼); RAST 3 and 4 (●).

Correlation between skin tests, inhalation tests and RAST results

Figs 8 and 9 show the comparison of the three methods in fifty-one subjects for house dust allergen and on forty-five subjects for *D. pteronyssinus* purified extract. The comparison of the two figures demonstrates that RAST result for house dust is often negative although the skin and inhalation test results are highly positive. On the other hand, discordant results with *D. pteronyssinus* extract are infrequent, subjects having highly positive skin test reactions and high bronchial sensitivity also having a high class of RAST score (3 or 4); only two patients had a negative RAST.

Discussion

The present study was carried out on selected patients who had positive skin test reactions to house dust and *D. pteronyssinus* allergens. However, among asthmatics with positive skin test reactions to house dust, some have negative skin test reactions to the house dust mite *D. pteronyssinus*. In these patients sensitivity to other allergens, such as animal epithelia and moulds, is frequently observed (Aalberse, Hoorweg & Reering-Brongers, 1975; Bessot *et al.*, 1976; Johansson, 1975; Virchow, Roth & Möller, 1976; Voorhorst & Spijksma, 1975).

House dust extracts must be considered a mixture of numerous allergenic components of varying composition, depending on their source. The weak concordance

between skin test and RAST for house dust could be explained in our study by differences in the non-standardized house dust extracts used in the two tests.

On the other hand, the close correlation between skin tests and RAST for *D. pteronyssinus* in the selected patients allergic to house dust and *D. pteronyssinus* demonstrates that the latter contains the antigenic determinants to which the subjects are sensitized. It is probable that the house dust extract fixed to the discs in the RAST technique does not contain a sufficient amount of the responsible antigens. It can be assumed that RAST for house dust is not of value in patients with house dust and mite allergy. Miyamoto *et al.* (1974) have reported a correlation between positivity of RAST results for *D. pteronyssinus* and for house dust, and that the values of specific IgE were generally higher for the *D. pteronyssinus* extract.

Our results with the *D. pteronyssinus* allergen extract confirm the findings of other investigators (Stenius & Wide, 1969; Stenius, Wide & Seymour, 1971; Bryant, Burns & Lazarus, 1975) who reported a significant correlation between the size of prick test reactions and the serum level of allergen-specific IgE. The results we obtained with the purified extract of *D. pteronyssinus* are comparable with those reported by Miyamoto *et al.* (1974), who demonstrated a close correlation between the level of specific IgE and the skin test threshold strength of allergen extract. However, in a few of the cases with positive skin test reactions, we were unable to detect specific IgE. Similar results have been reported in other investigations (Pepys, Roth & Carroll, 1975). Several explanations for this are proposed: (a) it is conceivable that, when the RAST result is negative, specific antibodies are fixed only on the mast cells, or (b) other investigators suggest the participation of different classes of antibodies, such as the short-term mast cells sensitizing IgG antibody, as described by Parish (1970).

Despite the limitations and imperfections of the inhalation tests, they nevertheless play an important role in assessing the possible clinical relevance of allergens causing positive skin tests or RAST. Inhalation tests may be erroneously positive if the inhaled allergen contains irritants capable of causing bronchospasm. This may be a possible explanation for the discrepancy between negative RAST results and positive inhalation test results.

A small number of patients give positive inhalation test results only with high dose of allergen, and yet have high levels of specific IgE in their serum. The affinity of the antibodies for the target cells and the competitive factors sometimes present in the serum may be responsible for these discrepancies (Vervloet, Fujita & Wypych, 1975).

Other investigators have found a strong correlation between the level of specific IgE and the IgE fixed to the target cells. Lichtenstein, Ishizaka & Norman (1973) in their studies of the level of specific IgE to ragweed and histamine release from basophils have demonstrated some concordance between these two tests. Nevertheless, individual variations exist between the level of IgE and cellular sensitivity. These variables are due to the complexity of mechanisms which are involved in the development of the allergic reaction, such as the number of mast cells, the affinity of the antibodies for the target cells, the receptors on the target cells, and regulatory factors which can play a role in the release of chemical mediators.

The correlation between RAST results and other *in vivo* test results depend on the quality and purity of the allergen extracts. Our findings with purified extract of *D. pteronyssinus* show that, given a highly selected group of subjects and purified allergenic extracts coupled to discs or used for cutaneous and inhalation tests, the correlation between RAST, inhalation and skin test results reaches 95%. However, the

bronchial provocation dose of antigen of purified mite extracts is probably much greater than the dosage encountered in a natural exposure.

The results of this study present relationships identical with those observed for other allergens such as ragweed (Norman, Lichtenstein & Ishizaka, 1973; Perera *et al.*, 1975). Nevertheless, we consider that the correlations we have observed between skin tests, RAST and bronchial sensitivity in *D. pteronyssinus* allergic patients raise many questions about the simultaneous use of these three methods of investigation in the routine diagnosis of *D. pteronyssinus* allergic asthma.

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