

Purification and Standardization of House Dust Allergens Based on Cutaneous Tests, RAST-Inhibition, and Complement Assay

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None of the methods proposed over the years for allergen standardization has so far proved entirely satisfactory (8). Furthermore, the divergent results produced by particular techniques with different allergens, e.g. grass pollen and house dust, have shown that the ultimate method of choice may be dependent on the nature of the allergen preparation to be standardized (3,4). For house dust extracts, comparative skin-testing (7), immunodiffusion-in-gel (3,9), RAST-inhibition (6) and complement (C) fixation have in the past been attempted as possible techniques of standardization.

In this investigation, four house dust allergen preparations of different degree of purification were examined in our various Institutes by means of physicochemical analysis, IgE binding, C-assay, and cutaneous tests. The philosophy of this approach was that, if a chosen technique has any validity at all in standardization, potency differences among various preparations should reveal themselves in an equal manner, independent of the investigator. The present paper gives an overview of the results. More detailed information will be provided in separate publications.

MATERIALS AND METHODS

Allergens

House dust extracts were prepared and purified according to a standard schedule, and subfractions were coded as published (9). Four different preparations, R₁—R₄, were distributed for multicentre investigations. Briefly, R₁ was a partially purified preparation from a large lot of pooled vacuum cleaner dust, purified to the stage of fraction C by benzoic acid adsorption and ammonium sulphate fractionation (1,3). R₂ was a relatively crude, benzoic acid adsorbed A-fraction from the dust of an ultramodern hotel (no pets allowed). From this product, a highly purified R₄ subfraction was prepared by purification to the stage of fraction E, followed by Sephadex G75-gel filtration and collection of the first peak off the column. The R₃-preparation was an ammonium sulphate precipitated fraction from a

direct house dust extract and was supplied by the Pasteur Institute in Paris.

RAST and complement assay

Preparations R₁ and R₄ were coupled to paper discs and used in direct (IgE) RAST and RAST-inhibition as reported elsewhere (6). Consumption of haemolytic C in patients' sera was measured either by kinetic assay (4), or was read from dose-response curves (2).

Skin tests

Skin tests were performed by the intracutaneous route in house dust-sensitive patients and were read by end-point titration with 10-fold serial dilutions (6).

RESULTS

The results of the *in vivo* and *in vitro* tests demonstrated that the various preparations had been well-chosen, because considerable differences in reactivity were noted. On comparing the relative potencies in RAST-inhibition—read from dose-response graphs with serum pools of at least 15 donors and employing either R₁- or R₄-discs—a potency sequence on average of R₁ > R₄ > R₂ > R₃ was established. A technological drawback was that determination of 50% inhibition point tended to be rather inaccurate due to large variations in duplicate measurements. Working with individual human sera, however, entirely different potency sequences were observed (6), indicating that the method can have only statistical predictive value.

Consumption of haemolytic complement by the R-preparations was either expressed in kinetic units (4), or was read as the 50%-C-consumption point from dose-response graphs (2). An example of the kinetic data is shown in Fig. 1. By way of the 50% consumption method, various laboratories—unaware of each other's results—agreed on an outstanding potency of the R₁-product, followed (or slightly exceeded) by R₄, with R₃ and R₂ as the least active preparations (Table 1). This sequence was established as the mean of several serum samples. However, it was noted that the sequence in in-

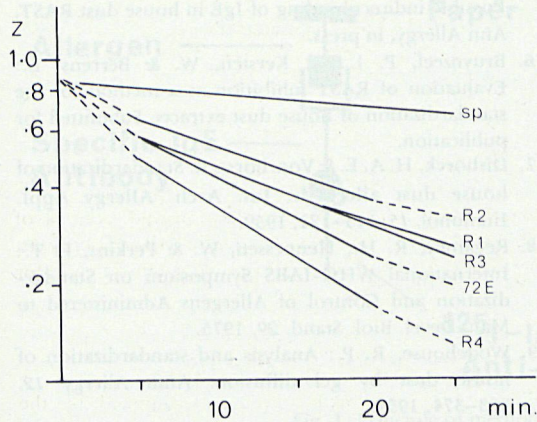


Fig. 1. Example of C-consumption in serum of an allergic patient (20 μ l, diluted 1:25) by the R-preparations. Conditions of the experiment as before (4). Quantities of lyophilized product used in the assay: 72E=1 μ g; R₁=0.5 μ g; R₂=4 μ g; R₃=3 μ g; R₄=1 μ g. Activity calculated from the slope of the time-curves after the first 5 min and expressed in units as discussed elsewhere (4). Taking the former standard 72E as 1000 U/ μ g, the relative potencies were: R₁=1470 U/ μ g, R₂=149 U/ μ g, R₃=254 U/ μ g. Corrected for spontaneous decay in the absence of allergen (sp.).

dividual serum samples on occasion was entirely different.

Intracutaneous tests in a randomized group of house-dust sensitive patients showed that this group could be divided into either strong or weak reactors. The strong reactors responded to a much lower allergen concentration (0.04 μ g R₁/ml) than the weak reactors. The dose-

response curves differed among individual patients and, markedly, among the various R-preparations. Evaluation of the results by end-point titration or from dose-response curves produced different figures (Table 1). From the data in the group of 16 strong reactors (with positive R₁-RAST values), the most active product again proved to be R₁, while the potency sequence was roughly similar to those established by RAST-inhibition or C-consumption (Table 1). As before, deviation from the mean was noted in some individual patients. With various subfractions from a single batch of house dust, the pattern of skin reactivity was similarly found to be subject to individual variation.

DISCUSSION

The data presented in brief in this paper demonstrate that skin-testing, RAST-inhibition and C-assay can all roughly predict a potency sequence for house dust extracts of different provenance or degree of purification. Although marked variation was noted in the potency ratios established among the different laboratories with these techniques (Table 1), there was an overall agreement on the products predicted to be most active. That this was true only in statistical terms was confirmed by each of the cooperating Institutes, because each reported numerical data in individual patients or sera which differed quite considerably from the mean. It would appear that this is a serious drawback in establishing standardization rules, because standardized allergenic products should be safe and have predictable potency for each and every patient attending an allergy clinic.

An interesting outcome of the investigation was that, although the potency ratios did not agree, the predicted

Table 1

Relative potencies and potency sequence of house dust preparations R₁, R₂, R₃ and R₄ in skin-testing^a, RAST-inhibition^b and C-consumption^c. Reference R₁ arbitrarily taken as unity

Assay ^d	R ₁	R ₂	R ₃	R ₄	Sequence
Threshold skin tests (4)	1.0	0.05	0.1	0.15	R ₁ > R ₄ > R ₃ > R ₂
Dose-response skin tests (4)	1.0	0.5	0.15	0.6	R ₁ > R ₄ > R ₂ > R ₃
Complement (1)	1.0	0.1	0.17	0.86	R ₁ > R ₄ > R ₃ > R ₂
Complement (2)	1.0	0.04	0.43	1.12	R ₄ > R ₁ > R ₃ > R ₂
Complement (4,5)	1.0	0.6	0.7	1.3	R ₄ > R ₁ > R ₃ > R ₂
Complement (6)	1.0	<< 0.1	0.25	0.6	R ₁ > R ₄ > R ₃ > R ₂
RAST-inhibition (1)	1.0	0.1	0.03	0.15	R ₁ > R ₄ > R ₂ > R ₃

^a Average values in a group of 16 patients.

^b Mean of two serum pools (15 sera per pool), R₁-discs.

^c Kinetic experiments (laboratory 1) or 50% consumption points from dose-response graphs (other laboratories); mean of 10 samples.

^d Numbers in parentheses refer to laboratory in conformity to author allocations indicated in the title of this article.

potency sequences established by RAST-inhibition and C-assay displayed some parallelism (Table 1). This finding is in concert with other data from C-assay, skin tests and direct RAST (5) which suggest that, although IgE- and C-mediated mechanisms in house dust allergic patients operate independently, there may yet be some association between these phenomena.

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Standardization of Radioallergosorbent Test (RAST) and Allergen Extracts in Terms of the WHO Standard for IgE

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Allergen extracts have been reported to vary considerably with respect to their potency as reflected by their ability to react in human skin (1,6). At the present time, there are no potency standards for such extracts and commercial preparations are listed in terms of either protein nitrogen per volume (PNU) or as weight percent of allergen extracted (being related to Noon units). Standardization is of course desirable, but progress towards this goal has been held back because of the lack of accepted criteria and the technology for measurement.

The use of skin testing for the measurement of potency of allergen extracts has been proposed (7), often linked with a histamine standard (2). In addition to the ethical and legal barriers to this approach, the limited number of suitable human volunteers makes it impracticable for implementation by a control authority

(3). Such *in vivo* techniques have only been practised in isolated clinics.

The development of the radioallergosorbent test (RAST) for the detection of specific IgE antibodies in the sera of atopic patients (8) has led to its adaptation for the measurement of allergens. The inhibition of the RAST by soluble allergen has now emerged as the method of choice for the standardization of allergen extracts.

The major problem of this approach is that RAST itself is not a standardized technique. In particular, it is not possible to directly compare two assays performed at different times because of variations in the binding of the labelled anti IgE. This problem has been approached by ranking atopic sera in the direct RAST (5) or by comparison with a reference allergen as inhibitors of the RAST (4).

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