

Autologous Dust RAST

A Neglected Tool to Detect Idiopathic Sources of Allergens in the Home

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Introduction

House dust extract has been used for many decades as a source of allergenic activity. In view of its variable composition (which makes it virtually impossible to standardize), its content of potentially infectious substances (e.g., material derived from human skin scales) and the discovery that the allergenic activity can to a substantial degree be attributed to products of a few well-defined organisms, the *in vivo* use of house-dust extract is discouraged.

However, the *in vitro* use of house-dust extract, particularly extracts of dust collected from the environment where the patient develops symptoms, can still provide information on idiopathic sources of allergens. The direct house-dust radioallergosorbent test (RAST) proves to be a potent tool for such an analysis.

Allergenic Activity of House-Dust Extract

The classical studies on the allergenic activity were based largely on association studies. Such studies clearly pointed to pyroglyphid mites as a major source of house-dust allergen (1,2). However, a more direct proof was obtained in the early 1970s by RAST-inhibition. This technology has been used in two distinct modes:

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1. House-dust extract has been used as an inhibitor of, e.g., the mite-RAST (3); this has indicated that house dust contains mite-related allergens, but does not indicate to what extent these mite-related allergens are accountable for the allergenic activity of the dust extract.
2. Specific allergen extracts (e.g., mite extract) have been used as inhibitors of the house-dust RAST (4,5). This approach directly indicates what fraction of the dust-reactive IgE is reactive with the specific allergen.

Technical Intermezzo: Use of House Dust Extract in the RAST

The use of the house-dust RAST is clearly the most direct way of accounting for the allergenicity of dust extract. However, it depends on the reliability of the use of an ill-defined material (house-dust extract) in a sophisticated and interference-prone test such as the RAST. Two very different problems were encountered, both related to the problem of working with micrograms of allergen hidden in grams of rubbish (a typical needle-in-a-haystack or one-in-a-million problem). The first problem was: the limited capacity of the solid phase. The second problem was nonimmune binding of IgE to the excess of non-allergenic material.

The capacity of the allergosorbent needs to be huge. Studies with purified allergens indicate that approx 10–100 ng allergen per test usually results in acceptable efficiency of IgE antibody binding. If the dust contains 1 µg allergen per gram of dust and if the total amount of protein extractable from 1 gram of dust is 1–10 mg, then 10–1000 µg protein should be coupled to the amount of solid phase used per test. An ELISA plate will usually bind <0.1 µg protein per well, but porous hydrophilic polysaccharides such as agarose may bind up to 100 µg protein per 10 µL gel. We have indeed found CNBr-activated Sepharose to be an excellent material to prepare allergosorbents for use in the house-dust RAST. In many cases the efficiency can be improved two to five-fold by a crude physicochemical fractionation of the dust extract, for example, by applying the extract in the presence of 1 M ammonium sulfate to a column of phenyl-Sepharose (1 mL gel/10 mL dust extract). After washing the column with 1 M ammonium sulfate, most allergenic activity can be eluted with water. However, such a fractionation procedure may not be appropriate for every allergen.

Nonimmune binding of IgE to the allergosorbent was often found to be substantial (3). This was tested with two purified IgE myeloma proteins (ND and PS) and IgE-rich serum from a filaria patient Ch, all three tested at 500 IU/mL. This IgE binding was clearly dependent on some dust component(s), as it could be inhibited by preincubation of

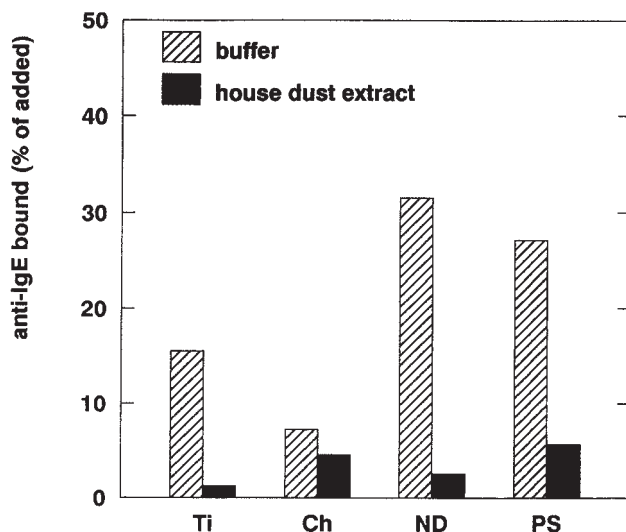


Fig. 1. Both specific and nonspecific binding of IgE to house dust extract is inhibitable by preincubation with house dust extract. Serum Ti contains IgE to cat allergen (and thus indicates specific binding of IgE); the other three samples are: serum of a filaria patient (Ch) and two myeloma proteins (ND and PS). All samples were diluted to a total IgE level of 500 IU/mL of IgE; 50 μ L sample was used in a final volume of 500 μ L.

the IgE-containing sample with dust extract (Fig 1). When we tested various detergents, buffers, and protective colloids, we found that addition of the positively charged polypeptide protamine (0.1 mg/mL) largely prevented this nonimmune binding; poly-L-lysine had a similar effect, but arginine or lysine was ineffective. We then tested other poly-cations and found that low-molecular weight (MW) polyamines such as dipropylene triamine (DPT) and triethylene tetramine (10 mM, pH 7.2) could also be used to partially prevent nonimmune binding. The efficacy of these low-MW polyamines could be enhanced by treatment with a crosslinker (epichlorohydrin: 1.8 molar equivalent added to a 0.1 M solution of the polyamine in 1 N NaOH at room temperature, 24-h reaction time). The selective effect of adding epichlorohydrin-treated DPT (EPA33; epichlorohydrin-treated polyamine [the digits 33 reflect the number of carbons separating the nitrogens in DPT]; we use it at 0.01 M DPT-equivalent, pH 7.2) on nonimmune and immune IgE binding is illustrated in Fig. 2. It is to be noted that in the case of nonimmune binding of myeloma IgE or filaria-specific IgE, IgE bound to house-dust allergosorbent in the absence of polyamine could not be removed by washing with 1 M NaCl or 1 M urea. This EPA33-containing buffer is used in all house-dust RAST assays presented here.

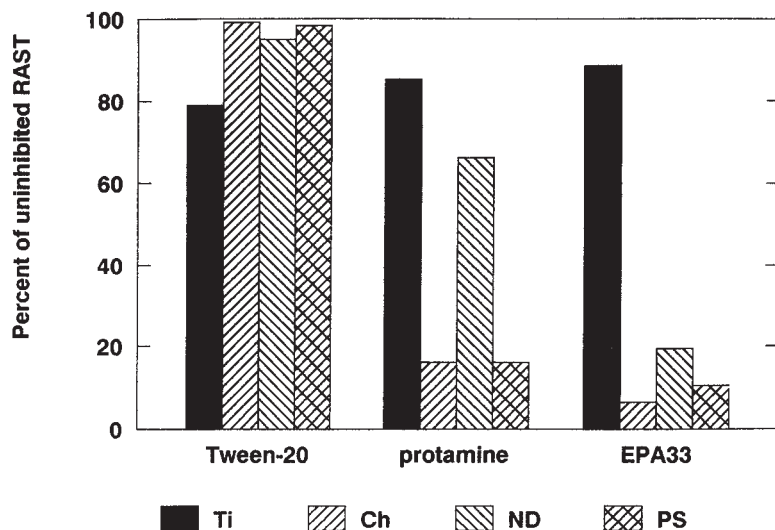


Fig. 2. Nonspecific binding of IgE to house-dust extract is inhibitable by polycations (protamine, EPA33), whereas specific binding of IgE (serum Ti) is not affected. As in the experiment shown in Fig. 1, all samples were diluted to a total IgE level of 500 IU/mL.

Inhibition of the House-Dust RAST by Specific Allergens

When we used pooled house dust as allergen source, there was a clear, but imperfect, correlation between the house dust RAST and the Dp-RAST. In the experiments shown in Fig. 3, we used serum samples from 10 patients. These sera were tested in a dilution that resulted in a similar score in the house-dust RAST. The house-dust RAST with the sera with the highest Dp-RAST at this dilution proved to be most efficiently inhibited by Dp extract, whereas inhibition by Dp extract was low when the Dp RAST was low. In the majority of the samples, the major additional IgE-binding components in the pooled dust extract proved to be cat allergens (Fig. 3, Table 1) (5). For the majority of the sera, a mixture of mite and cat extract mimics the reactivity of house dust extract (Fig. 4). Other furred pets (dogs, rabbits, and guinea pigs) were also found to contribute to the IgE binding activity of this pooled dust extract. In some dust samples, significant quantities of pollen allergens were found. In addition, cow's milk protein and wheat protein, and unexpectedly even chicken egg white, were found to be present in quantities sufficient to cause substantial RAST-positivity (6).

We confirmed these results using individual dust extracts. Using the direct house dust RAST (and confirmed by inhibition of the house dust RAST), allergens from cats and dogs were found in dust samples

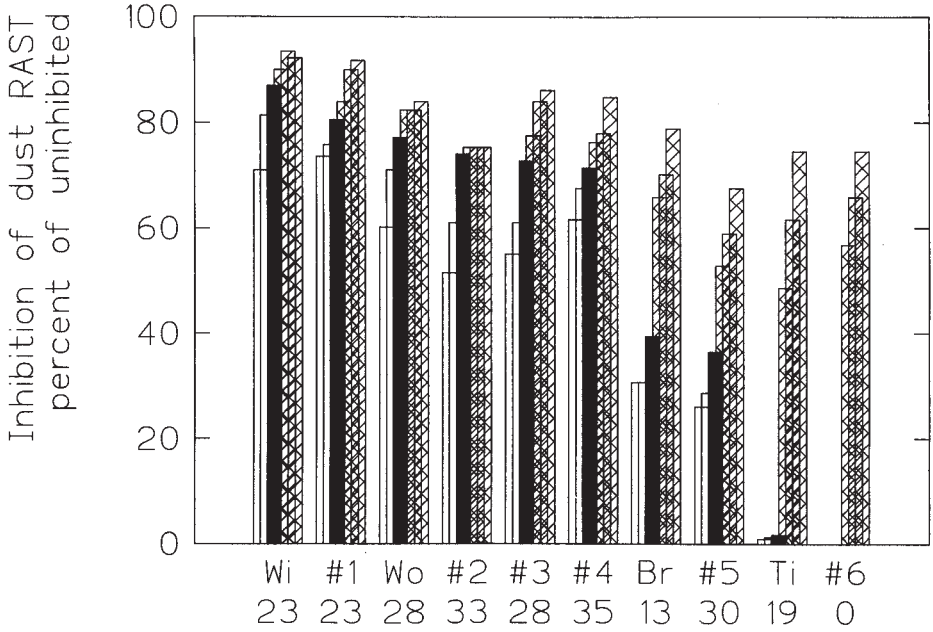


Fig. 3. Inhibition of the house-dust RAST by mite extract (threefold increasing doses, two open bars and filled bar); also, threefold increasing doses of cat-dander extract were added (hatched bars) to the highest dose of mite extract. The numbers below the sample identifications indicate the RAST score to house dust mite (expressed as percentage of anti-IgE bound).

Table 1
Inhibition of House-Dust RAST
by a Combination of *D. pteronyssinus* Extract,
Cat-Dander Extract, and Dog-Dander
Extract in Comparison with Inhibition
by House-Dust Extract^a

Inhibitor	Ti	Br	Wi
None	100	100	100
House-dust extract	24	25	19
Mite	100	79	29
Cat + dog	16	39	99
Mite + cat + dog	17	13	20

^aTi, Br, and Wi are three sera. The results are presented as percentage of uninhibited RAST.

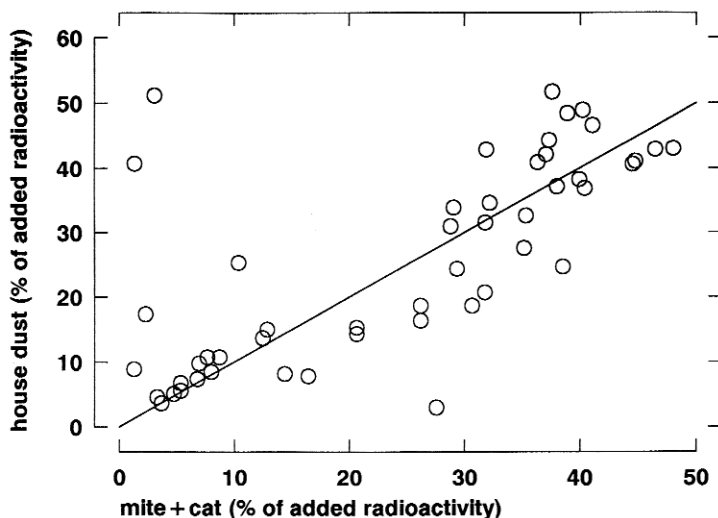


Fig. 4. RAST results obtained with mixture of mite and cat extract in comparison with the RAST of pooled dust extract. The line shown indicates identity.

even from houses without these pets (3); this was not so for allergens from less common pets such as rabbit and guinea pig. We tested over 100 serum samples from patients reactive in the RAST with pooled house dust extract and found that more than 80% of the reactivity of more than 90% of these sera can be accounted for by these four groups of allergens (mites, furred pets, common foods, pollen).

Strikingly, we did not find support for the notion that mold allergens were major contributors to the IgE binding activity of house-dust extract. The problem of crossreactive allergens, particularly from invertebrate origin, will be dealt with in the next section.

The Problem of Crossreactivity, Particularly between Mites and Other Invertebrates (e.g., Cockroach, Silverfish)

IgE reactivity to mites is invariably equated with exposure to house dust mites. This is usually likely to be the correct interpretation. It is important to appreciate, however, that IgE reactivity can also be induced by crossreactive allergens. This is particularly relevant in the case of IgE to invertebrate-derived material, because crossreactivity is relatively common. One highly crossreactive allergen is the muscle protein tropomyosin (7), but this is certainly not the only source of crossreactivity among invertebrates.

Our results indicate that IgE reactive with extracts of a wide variety of invertebrates (e.g., cockroach, silverfish, chironomid, book louse,

and often even shellfish) is relatively common in sera with IgE to Dp., but (at least in The Netherlands) uncommon in sera without IgE reactive with Dp. The results of RAST inhibition experiments indicated that most (but not all) reactivity in these sera is attributable to IgE to mite-related allergens (8).

Because silverfish is common in the Dutch indoor environment, we were interested in determining exposure and thus developed an immunoassay. We have not yet purified a purified silverfish antigen, so we presented our results in total extractable silverfish protein (9).

Relevance for the Patient of the Finding of IgE Reactive with Silverfish

There are at least two related, but still distinct questions. One question is the source of the sensitizing allergen: is it really silverfish or is it a dust mite (or perhaps another invertebrate species)? Unless the sensitizing species is unknown, this question can to a large extent be answered by reciprocal RAST inhibition; the extract that results in the most complete and efficient inhibition of the silverfish RAST is the most likely candidate for the sensitizing species. Another question is: is current indoor exposure of the patient sufficiently high to cause symptoms? When both an assay and a reference based on a purified allergen is available, a comparison with other indoor allergens can be made; this analogy with other indoor allergens would indicate that allergen levels higher than 1000 ng/g fine dust are likely to cause symptoms, whereas levels below 100 ng/g dust are unlikely to do so. When the quantitative estimation of allergen levels in house dust is not yet possible, a semiquantitative estimate can be based upon the results of the autologous house-dust RAST; if this test with patients own serum is clearly positive, analogy with the mite allergens indicates that the allergen level is sufficiently high to cause symptoms. It is obviously essential to remove IgE reactive with the common indoor allergens (mite, cat, and so on) by prior absorption, preferably using solid-phase coupled allergens. These studies still need to be done in the case of silverfish.

Idiosyncratic Indoor Allergens

The description "idiosyncratic" is used to cover two different issues: on the one hand patients with a rare sensitization to a common allergen, and on the other hand, patients with a sensitization to an allergen that is only rarely found in the indoor environment. In both cases, the house-dust RAST is useful. In the latter case, an autologous dust extract is clearly essential. However, even in the first case the

autologous RAST (in addition to, or in combination with, RAST inhibition) may be very helpful to support the claim that indoor exposure is likely to contribute to symptomatology. The aforementioned example of patients with IgE reactive in the silverfish RAST may serve as an illustration.

We approached the search for idiosyncratic allergens in house dust in various ways. One important aspect is the source of the sera used for screening. One panel consisted of sera from patients suspected to have allergic airway symptoms but no IgE to any of the known indoor allergens, but yet an elevated total IgE (200–1500 kU/mL; sera with a higher total IgE were excluded in view of potential problems with nonspecific binding). Another panel consisted of sera from patients with IgE to Dp. but not to any other known indoor allergen; reactivity to mites was removed by absorption with Dp-extract coupled to Sepharose. In both panels we found sera with IgE reactive with pooled house dust. The most intriguing result was obtained with serum from a patient Ka, with perennial rhinitis as the main complaint. When we tested this serum (total IgE: 410 IU/mL, specific IgE to weed pollen, but not to known indoor allergens) in the house-dust RAST, this resulted in more than 20% of added anti-IgE bound to 2 out of 10 individual dust extracts. When we tested autologous house dust, the RAST result was even more striking: 60% of added anti-IgE was bound. A connection with the weed-reactive IgE was unlikely. Not only was the level of reactivity to weed substantially lower, but also no crossinhibition between the dust extract and the weed extract was found, and changes in IgE titers in blood samples obtained on different dates were not correlated. Using inhibition of the autologous dust RAST as an allergen assay, we were able to establish the distribution of allergen in the house and looked for seasonal variation (which we did not convincingly find). The source of this enigmatic allergen is still unsolved, even though we and others have tested well over 100 likely and less likely candidates.

Acknowledgments

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their store of allergens and confirming the exceptional reactivity of this serum to selected dust samples.

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