

Photo-inactivated allergens

II. Clinical experience with house dust allergen in pulmonary function tests and in immunotherapy

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Summary

Purified house dust allergen irradiated with UV-light loses its capacity to elicit immediate type skin and bronchial provocation reactions in the majority of asthmatic patients. However, in some patients both immediate and/or late bronchial reactions were observed to the active house dust and its inactivated counterpart.

Immunotherapy with UV-irradiated house dust allergen, which hyposensitized 66% of forty-eight asthmatic patients allergic to house dust, was as effective as treatment with aluminium oxide-adsorbed active house dust, and there were fewer adverse side-effects.

Introduction

Hyposensitization with atopic allergens is an ill-understood and complex process. With house dust extracts, variability has been observed by several authors as regards the efficacy of different commercial preparations (Aas, 1966; Baer *et al.*, 1970; Foucard *et al.*, 1972) as well as in the response of individual patients, depending on a 'high' or 'low' atopic status (Pepys, 1969).

In an attempt to standardize the procedure of house dust immunotherapy, house dust allergen has been purified and has been adsorbed onto aluminium hydroxide (Berrens, 1971; Hénocq, Relyveld & Raynaud, 1965). Thus, a stable, standardized preparation became available, which was used in therapeutic trial at three different concentration levels, i.e. low, medium or high, injections of standard volumes being performed by the subcutaneous route and at fixed intervals. The results of controlled immunotherapy of this kind have been previously reported (Hénocq *et al.*, 1965; Relyveld *et al.*, 1968).

The availability of a partially purified house dust allergen preparation and its photo-inactivated counterpart (Berrens, Hénocq & Radermecker, 1973) has led us to seek an answer to two major questions:

- (1) Is *allergenicity* of house dust allergen, i.e. capacity to produce weal and

erythema skin reactions or bronchial reactivity in allergic subjects, an essential prerequisite for hyposensitization of atopic patients?

(2) Does photo-inactivated house dust allergen retain its immunogenicity, sufficient to induce protection against the clinical manifestations of house dust atopy?

The present report describes preliminary attempts to answer these questions.

Materials and methods

Allergens

A stock solution of partially purified house dust extract, fraction HEC (Berrens, 1971) at 0.25 mg/ml was used together with its derivative HEC/UV at the same concentration and obtained after 30 min of UV-irradiation as described in the foregoing paper (Berrens *et al.*, 1973). Both preparations were employed for bronchial provocation tests (by inhalation) and for skin tests in asthmatic patients.

Inhalation tests

The allergen solutions were aerosolized from an 'Aerosolan' nebulizer (Tiffeneau, 1957), type Godart-Statham, Bilthoven (Netherlands). An air pressure of 0.80 kg/cm² was applied to the nebulizer. The aerosolized allergen was produced by passing air through the nebulizer at a rate of 20 l/min. One litre of aerosol comprised a volume of 0.01 ml of liquid phase, and roughly carried 12.5 µg of HEC or HEC/UV. The buccal tip was connected; (a) to a two-way valve, allowing the inhalation of the nebulized solution, and its rejection during the expiration period; (b) to the spiograph (Godart-Statham, Bilthoven) by means of a two-way tap.

Measurement of FEV₁ was performed before and 20 min after each inhalation. A fall of 10–20% of the FEV₁ value was considered significant. Bronchopulmonary allergy was appreciated as being:

- (i) very obvious at a threshold dose of 10 and 100 µg of allergen;
- (ii) obvious at doses between 100 and 500 µg,
- (iii) detectable between 500 and 2000 µg, and
- (iv) non-detectable at dosages over 2000 µg.

Late bronchial reactions were observed between 7 and 24 hr after the inhalation test.

Skin tests

Prick tests were performed with a 1000 PNU solution of *Dermatophagoides farinae* house dust mite extract, and with a 5000 PNU solution of house dust extract (both from Dome Laboratories, Slough, Great Britain). Tests with the active house dust preparation HEC and its ultraviolet irradiated derivative HEC/UV were performed by the intracutaneous route, using solutions at 2 µg/ml.

Patients

Asthmatic subjects who had previously been submitted to skin tests, inhalation tests, and measurement of FEV₁, FVC, FEV₁/FVC, methacholine challenge, and assessment of the reversal reaction by the inhalation of 200 µg of Salbutamol (Glaxo Laboratories) were treated:

- (a) by immunization with increasing concentrations of photo-inactivated house

dust, ranging from 0.2 to 2.0 $\mu\text{g/ml}$, by subcutaneous administration twice a week; this schedule was modified to once a week or every 2 weeks if aluminium hydroxide-adsorbed allergen suspensions were applied (2% Al_2O_3 , allergen concentration 2–20 $\mu\text{g/ml}$);

(b) by subcutaneous injections of HEC/UV subsequent to a preceding hyposensitization course with a different house dust preparation (aluminium oxide-adsorbed purified house dust 'Alipagène', Pasteur); the injections were given every week or every other week;

(c) by treatment with 'Alipagène' house dust at the dose and interval schedule reported before (Hénocq *et al.*, 1965; Relyveld *et al.*, 1968).

Each patient was kept under regular control every 6 weeks during therapeutic trial, other medication being continued or, in the case of progressive improvement, being discontinued at the patient's own request. This study has covered a period of 1 year.

Results

(1) Ventilation tests

Table 1 shows the results of skin- and inhalation tests in a group of thirty asthmatic patients, aged 11–75; all had perennial asthma, attributed clinically to house dust allergy. All of these patients presented positive intradermal tests to house dust HEC and positive prick tests to commercial house dust, *D. farinae*, or both. Two exceptions were noted, who were positive to HEC, but did not react to the prick tests; one of them (no. 13) produced a negative inhalation-provocation test, but the other (no. 17)—though displaying a very poor skin test response to HEC—reacted by inhalation to a minute quantity (200 μg) of HEC.

The skin-reactive potency of the photo-inactivated dust allergen HEC/UV was very unimpressive, only five out of thirty patients responding; likewise, only four patients continued to react to inhaled inactivated allergen. Four patients who experienced no immediate bronchial reaction to the active product HEC developed a late bronchial reaction some hours after the inhalation.

The minimal effective concentration of dust HEC for provoking a significant fall of the FEV_1 did not always run parallel to the intensity of the skin reaction. This is illustrated by patients 1, 4, 19, 22 and 8, where significant skin-reactions were associated with low bronchial reactivity. Conversely, in case No. 8, unimpressive or undetectable skin-reactivity occurred in combination with high sensitivity to bronchial challenge with both HEC and HEC/UV.

Table 2 gives a comparative list of the results of ventilatory tests in sixteen asthmatic patients before and after bronchial challenge with HEC and its inactivated counterpart HEC/UV. The fall of the FEV_1 with HEC, but not with HEC/UV, was significant in every case and, in the six patients studied, was comparable in magnitude to the methacholine challenge. The reversal reaction to pre-test level by inhalation of Salbutamol was significant in all cases examined.

Table 3 presents the results of a comparative investigation of six asthmatic patients with positive bronchial provocation reactions to both HEC and HEC/UV. In these cases, skin-reactions to the photo-inactivated allergen HEC/UV were poor or absent. In each of these patients, bronchial reactivity to the irradiated dust allergen had diminished in comparison to the active parent product HEC.

Table 1. Comparison between skin-tests (prick and ID) and inhalation tests with active purified house dust allergen HEC and photo-inactivated house dust HEC/UV. +, ++, +++: 1, 2 or 3 cm of the weal and flare reaction, respectively

Case	Intracutaneous tests			Prick tests		Inhalation tests (minimal effective concentration of allergen in μg)		Comments
	HEC 2 $\mu\text{g}/\text{ml}$	HEC/UV 2 $\mu\text{g}/\text{ml}$	House dust	<i>D. farinae</i>	HEC	HEC/UV		
			5000PNU	1000 PNU				
1	++	0	++	++	750	0		
2	++	++	+	++	100	0		
3	++	0	++	++	0	0	Hyposensitized	
4	++	+	++	++	1000	0		
5	++	0	0	++	0	0		
6	++	0	++	++	0	0		
7	++	0	++	+	500	0		
8	+	0	++	++	50	150		
9	++	0	0	++	0	0	Late bronchial reaction (HEC)	
10	++	0	0	++	300	N.D.		
11	++	0	+	++	0	0	Bronchial reaction to pollen (200)	
12	++	0	+	++	0	0	Late bronchial reaction (HEC)	
13	++	+	0	0	0	0		
14	++	0	++	++	0	0	Late bronchial reaction (HEC)	
15	++	0	++	++	100	N.D.		
16	++	0	0	++	0	0	100 with crude extract	
17	++	0	0	0	200	0		
18	+	0	+	++	100	0	Late bronchial reaction (HEC)	
19	++	0	++	++	1000	0		
20	++	0	+	++	0	0	Infective asthma	
21	++	0	+	++	200	0		
22	++	0	++	++	1000	0		
23	++	0	++	0	750	N.D.		
24	++	0	++	++	600	0		
25	++	0	++	++	500	1000		
26	++	+	++	++	200	500		
27	++	0	0	+	250	1500		
28	++	0	++	++	750	0		
29	++	0	++	++	750	0	Hyposensitized	
30	++	+	++	++	0	0		

N.D. = Not done.

Table 2. Bronchial challenge with purified house dust HEC and its ultraviolet-irradiated product HEC/UV in sixteen asthmatic patients

FVC:	3300	4860	2010	4200	2610	4860	2610	2850	3180	2910	2730	2790	5700	4990	4950	4650
FEV ₁ :	1680	3690	1770	2940	1050	3060	1380	2130	1800	1800	1470	1560	3690	3120	3570	3600
FEV ₁ — (°):	50.5	76	88	70	40.5	63	53	75	53.5	62	54	56	64.5	63	71	78
FVC																
Methacholine (°)			-17		-18			-16	-20	-25						-15
After HEC (°)	-25	-25	-20	-16	-20	-16	-20	-25	-25	-20	-25	-18	-22	-12	-12	-20
After HEC/UV	0	0	0	0	-20	0	0	0	0	0	-12	-12	-10	0	0	0
After Salbutamol (reversal reaction to pre-test level)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N.D.	+

N.D. = Not done.

Table 3. Skin and inhalation tests in six asthmatic patients with positive bronchial tests to both active (HEC) and photo-inactivated (HEC/UV) house dust allergen

Skin tests (intradermal, 2 µg/ml)		Inhalation tests (minimal effective concentration of allergen, in µg)	
HEC	HEC/UV	HEC	HEC/UV
1 +++	0	250	500
2 +	0	50	150
3 ++	0	1000	1500
4 +++	0	500	1000
5 +++	0	250	1500
6 +++	+	250	1500

(2) Immunotherapy

(a) With photo-inactivated house dust HEC/UV. The results of immunotherapy in forty-eight asthmatic patients with photo-inactivated dust allergen HEC/UV have been evaluated in Table 4. Patients who had received subcutaneous injections during a 6 week course were considered to be completely improved if they had renounced, at their own initiative, the use of subsidiary treatment, i.e. disodium cromoglycate, corticosteroids, or aminophyllin; they were classified as partially improved if they expressed relief of their symptoms without, however, discontinuation of symptomatic medication.

Though different therapeutic schedules were attempted, the relative number of patients relieved of their symptoms proved about constant, whatever the dosage applied (Table 5). Ten patients (Table 5, third column) experienced adverse asthmatic reactions to increasing doses of HEC/UV, though eventually three out of this group were clinically improved by immunotherapy with the inactivated allergens.

A second group of fourteen patients was treated with HEC/UV adsorbed to aluminium hydroxide, at 2 or 20 µg/ml. These patients received 6 subcutaneous injections of 0.1 ml every fortnight over a 12-week period. Within this group, eleven subjects were clinically improved.

(b) Table 6 gives the results of immunotherapy with HEC/UV subsequent to a hypo-sensitization course with a different preparation of house dust adsorbed to aluminium oxide ('Alipagène'). When these patients consulted for a relapse of asthma, immunotherapy with HEC/UV was instituted; five out of thirty-one subjects responded favourably to the inactivated material.

(c) For comparison, a group of thirty-eight asthmatic patients was treated with active house dust-aluminium oxide mixture 'Alipagène' in a schedule similar to that dis-

Table 4. Results of immunotherapy with photo-inactivated house dust HEC/UV (forty-eight asthmatic patients treated)

Totally improved	Partially improved	Not improved	Total
28	8	12	48

Table 5. Doses employed for immunotherapy with house dust allergen irradiated with ultraviolet light (HEC/UV)

	HEC/UV, 2 μ g/ml, 0.1-10 ml in 10 doses; course 6 weeks	HEC/UV, 2 μ g/ml, 1 ml in 10 doses; course 6 weeks	HEC/UV, 2 μ g/ml, 0.1 ml $\rightarrow$ adverse reaction in 10 doses; course 6 weeks	HEC/UV, 0.2 μ g/ml, 0.1-1 ml in 10 doses; course 6 weeks	HEC/UV, 20 μ g/ml, 0.1 ml in 10 doses; course 6 weeks
Improved	6	16	3	4	7
Not improved	1	1	7	2	1
Total	7	17	10	6	8

Table 6. Results of immunotherapy: comparison between 'Alipagène' and photo-inactivated house dust HEC/UV

Better with HEC/UV	Comparable improvement	Comparable failure	Comparable adverse reactions	Total
5	17	7	2	31

cussed under (a). Of this group, twenty-three subjects were completely relieved, ten patients recovered only partially, and in five individuals the treatment had no effect. These figures are in confirmation of previously reported results (Hénocq *et al.*, 1965; Relyveld *et al.*, 1968).

With the above three groups of patients, immunotherapy was continued over a 1 year period; each patient was recalled for control every 6 weeks.

Discussion

The results of these studies show that the majority of our asthmatic patients did not respond any longer to skin or bronchial tests with the photo-inactivated dust allergen HEC/UV. They could, therefore, be immunized with this product without any untoward reaction. The therapeutic results showed that 66% of the groups of patients had been totally or partially relieved of their symptoms by this variant of immunotherapy. Compared with hyposensitization with active house dust 'Alipagène' the results were identical. The first question raised in the Introduction can thus be answered in the sense that allergenicity, in the sense of detectable skin or bronchial reactivity of the allergen, does not appear to be essential in immunotherapy with house dust.

The question of unimpaired immunogenicity can less easily be answered. We observed six patients who reacted to both active HEC and irradiated HEC/UV, though the skin reactivity to the latter was poor or absent and the potency of HEC/UV in bronchial challenge had fallen by a factor between 1.5 and 6 (Table 3). Skin reactivity to both HEC and HEC/UV in one of these patients (no. 6, Table 3) could be passively transferred by a Prausnitz-Küstner reaction. However, the response reaction of the recipient to challenge with HEC or HEC/UV was a late reaction, occurring 4-6 hr after the challenging allergen injection. This patient experienced a dual type bronchial reaction to the inhalation of the allergens: the fall of FEV₁ was prolonged in time and the asthmatic attack lasted over 24 hr after the provocation test. Attempted hyposensitization with HEC/UV, in whatever dose, remained unsuccessful and was accompanied by severe local and bronchial reactions. This kind of response (including type I and III reactions) has been reported before with other allergens, e.g. *Aspergillus* (Pepys, 1973).

Two other asthmatic subjects presented dual type I and III skin reactions to active HEC; this was associated with a positive bronchial challenge (dual type I and III), but with inactivated HEC/UV only a late (type III) bronchial reaction was observed.

In view of these observations, a complex situation arises, in that several different mediators seem to be involved, viz: (a) a complement-dependent mechanism triggered *in vitro* by the 'allergenic' lysine-sugar sites, and which, though it possibly plays a role in *in vivo* allergic reactions, does not appear essential for immunotherapy; (b) an

immediate type specific antibody, probably IgE, directed against specific antigenic determinants and mediating the type I positive PK reaction; (c) a type III reaction inducing specific antibody of some other class mediating the late PK and bronchial reactions. It must remain undecided whether the latter could be identical to the recently described 'short-term' IgG anaphylactic antibody (Parish, 1970).

May *et al.* (1970) have shown that hyposensitization with atopic allergens requires high dosages, though the intensity of treatment may depend on the purity of the allergen (Aas, 1972). Our results in thirty-eight patients (Table 5) show that the effect of the treatment was independent of the dosage or the concentration schedule. If the results of immunotherapy are not dose-dependent, we feel that the possible rise of IgG 'blocking' antibody titre cannot be the only mechanism involved in protection. Indeed, modification of both cellular and humoral factors may also be important factors in the process of hyposensitization (Brostoff & Roitt, 1970; Levy & Lichtenstein, 1970). In view of these considerations, we are unable as yet to provide an answer to the second question raised in the Introduction to this paper.

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