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House dust extracts contain potent immunological adjuvants

Cees J. Beukelman[1], Hans van Dijk[2], Piet C. Aerts[1], Pieternel M. Rademaker[1], Lubertus Berrens[2] and Jan M. N. Willers[1]

[1]*Immunology Section of the Laboratory of Microbiology, State University, Catharijnesingel 59, Utrecht, The Netherlands, and* [2]*Division of Experimental Allergy, University Hospital, Catharijnesingel 101, Utrecht, The Netherlands*

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1. Summary

A crude aqueous extract of house dust and two house dust subfractions were tested for adjuvant activity in a sensitivity assay performed in mice. Evidence is presented that house dust contains at least two potent immunological adjuvants. One of these, present in both subfractions, was probably endotoxin and acted in a complement-independent way. The immunostimulatory effect of the other adjuvant was abrogated by prior complement depletion of the animals. This apparently complement-dependent adjuvant needs further identification.

2. Introduction

House dust may be considered as a complex mixture of organic debris and particles of mineral and synthetic origin. It contains a great number of antigenic or, more particularly, allergenic epitopes which are also present in a crude aqueous extract (fraction Ac). Upon incubation with fresh human serum this extract activates the classical complement pathway [6, 7, 10]. On the basis of skin reactivity in a representative group of atopic individuals fraction Ac has been purified by benzoic acid adsorption and differential precipitation with ammonium sulphate and by pH variation [8]. Throughout the purification procedure the skin-reactivity was paralleled by anti-complementary activity [5], suggesting that there might be a causal relationship between complement-activation and antigenicity or allergenicity. Such a relationship is compatible with a proposed role of complement in the induction of IgM-, IgG- and IgE-responses [17–19]. The involvement of complement in these responses might be an indirect one, e.g. by providing a second signal for B cell activation [9]. The effect of complement could also be mediated by substances with, for example, adjuvant activity present in the antigenic or allergenic preparation. The latter is in line with findings of Klerx et al. [12–15, 20] suggesting that complement is involved in immunological adjuvant activity.

In a recent publication [3] it was shown that the most allergenic fraction derived from house dust (fraction C) also activated the mouse classical complement pathway. This implies that the mouse is suitable to investigate the relationship between complement activation by house dust fractions and their adjuvant activity in the allergic response.

The IgE response as a secondary immune reaction is necessarily preceded by a primary reaction through the production of IgM antibodies. For this reason the effects of house dust preparations on the IgM-response were studied first. For these experiments an adjuvant assay, based on the immunogenicity of neuraminidase-treated sheep red blood cells (asialo-SRBC), was used [20].

Key words: Adjuvants; Immunological; Allergy; Complement; House dust

Correspondence to: C. J. Beukelman, Immunology Section of the Laboratory of Microbiology, State University, Catharijnesingel 59, 3511 GG Utrecht, The Netherlands.

3. Materials and Methods

3.1. *Animals*

Male BALB/c mice reared in our Institute were used at an age between 10 and 12 weeks.

Female F_1 (New Zealand White × Flemish Giant) rabbits obtained from the Central Institute for the Breeding of Laboratory Animals (CPB, Zeist, The Netherlands) served as donors of erythrocytes for the complement assay.

3.2. *Sera*

Blood obtained from mice by puncture of the retro-orbital plexus was allowed to clot at room temperature for 1.5 h. The clot was spun down and the serum collected. In the complement assay fresh serum was used; for the hemagglutination test the serum was heat-inactivated before use (56 °C; 30 min).

3.3. *House dust preparations*

House dust was collected in several animal-free houses by vacuum cleaning. The contents of the vacuum cleaning bags were brought into 0.028 M phosphate buffer, pH 6.9, containing 0.01 M NaCl and 0.5% phenol. The extract was filtered through a kieselguhr layer and dialyzed exhaustively against tap water and distilled water.

The non-dialysable fraction (Fraction Ac) was purified by benzoic acid co-precipitation and ammonium sulphate precipitation to the stage of fraction C [4]. The preparations were lyophilized and stored at 4 °C until use.

3.4. *Determination of adjuvant activity*

House dust fractions were tested for adjuvant activity by a sensitive in vivo assay using asialo-SRBC as antigen [20]. In brief: mice were injected i.p. with 0.5 ml of pyrogen-free saline containing 3×10^6 asialo-SRBC and the adjuvant. Seven days later the primary antibody response was measured by a microtitre haemagglutination assay. Titres were expressed as $-\log_2$ of the final agglutinating dilution. Dextran sulphate (M_r 500K; Pharmacia, Uppsala, Sweden) was used as reference adjuvant. Lipopolysaccharide (LPS) type W from *E. coli* 0111:B4 was obtained from Difco (Detroit, MI, USA).

3.5. *Estimation of endotoxin content*

The proportion of endotoxin contamination of house dust fractions was tested by Mr. F. Hagelen and Dr. F. C. Hillen (National Institute of Health and Environmental Hygiene, RIVM, Bilthoven, The Netherlands) using a microtitre variant of the Limulus Amoebocyte Lysate (LAL) assay [16, 21]. Pyrogenicity of fractions was determined in rabbits according to the European Pharmacopoeia (1985).

3.6. *Anti-complementary activity*

Consumption of mouse overall complement activity was measured using a microtitre assay [11] after preincubation of the serum with house dust fractions at 39 °C for 30 min. Checkerboard titrations were performed in which the serum dilution was varied in one direction and the dose of the putative anti-complementary substance in the other. The dose of the test substance causing a 50% decrease in complement activity at 50% hemolysis was designated ID_{50}-value.

3.7. *Precipitation with polyethylene glycol*

House dust fraction Ac was dissolved to a concentration of 5 mg per ml in pyrogen-free veronal (5 mM) saline buffer, pH 7.35, containing 0.15 mM Ca^{2+} and 0.5 mM Mg^{2+} (VSB^{2+}) and centrifuged (15000 × *g* at 20 °C for 10 min). The supernatant was diluted (1:10) with VSB^{2+} containing incremental amounts of polyethylene glycol 6000 (PEG; BDH, Poole, U.K.), incubated under repeated whirling at 4 °C for 30 min, and spun at 15000 × *g* (20 °C, 10 min). Precipitates were redissolved in VSB^{2+} to starting volume (5 mgequiv. per ml).

3.8. *Treatment with cobra venom factor*

The day before immunization mice received two i.p. injections, with an interval of 8 h, of 0.2 ml of pyrogen-free saline containing 20 microtitre units (±0.2 units, according to Ballow and Cochrane; [1]) of phospholipase-free cobra venom factor (CoF) prepared from *Naja naja* venom [2]. Control animals received heat-inactivated material (72 °C, 60 min; CoF*) or saline only. The treatment with the active material resulted in a total complement depletion for a period of five days.

3.9. *Statistics*

Results are expressed as the arithmetic mean of *n*

determinations ± the standard error of the mean (SEM). Student's *t*-test (two-sided) was used to estimate the significance of differences observed. Differences with *P* values greater than 0.05 were taken as not significant.

The coefficient of correlation (*r*) was determined by the method of least squares. The SE in any given point was calculated by the formula:

$$SE = \sqrt{\frac{\Sigma(Y_i - \bar{Y} - p(X_i - \bar{X}))^2}{n-2}} \times \left(\frac{1}{n} + \frac{(X-\bar{X})^2}{\Sigma(X_i - \bar{X})^2}\right),$$

in which X = x-value of the point of interest, X_i = x-value of a measuring point, $\bar{X}$ = mean of all X_i-values, Y_i = y-value of a measuring point, $\bar{Y}$ = mean of all Y_i-values, p = tangent of the linear regression line ($y = px + q$) and n = number of paired observations.

4. Results

The immunological adjuvant activity of house dust fraction C, a potent activator of the human [6, 7, 10] and mouse [3] classical complement pathways, was investigated and compared with that of dextran sulphate, the most active adjuvant tested in the asialo-SRBC assay so far [20]. As shown in Fig. 1, fraction C displayed dose-dependent adjuvant activity. On a weight basis, fraction C was about nine times less effective than dextran sulphate.

Since the adjuvant activity could be due to lipopolysaccharide, fraction C was tested in the LAL-assay and compared to the crude house dust fraction Ac in the same test. The results presented in Table 1 indicate that the apparent lipopolysaccharide content of fraction C was quite considerable. In the pyrogenicity test in rabbits, fraction C (1:100 dilution) was also more active than fraction Ac in similar dilution.

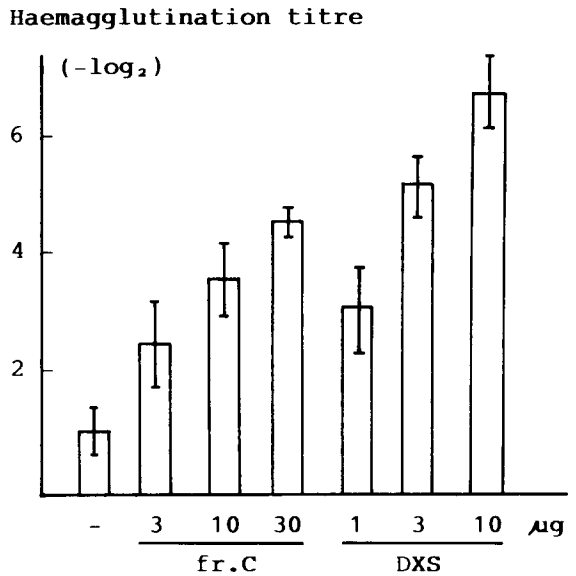

Fig. 1. Dose-dependent immunological adjuvant activities of house dust fraction C and dextran sulphate on the hemagglutinin response of mice ($n = 5$) to asialo-SRBC. Control animals received asialo-SRBC only.

To investigate whether the adjuvant activity of the house dust fractions may be ascribed to the presence of endotoxin, the potencies of fractions Ac and C were compared with that of commercial lipopolysaccharide. The same fractions and lipopolysaccharide were also examined for complement activation. Fig. 2 (left hand panel) shows a significant correlation between dose and adjuvant activity for lipopolysac-

Table 1
Data obtained for house dust fractions Ac and C.

	Endotoxin content LAL-assay (ng/mgequiv.)[a]	Pyrogenicity Δt (°C)[b]	Anti-complementary activity (ID_{50} value; ng)[c]
Fraction Ac	82 ± 28	0.56 ± 0.03	2100
Fraction C	1622 ± 351	1.23 ± 0.13	175

[a] $n = 3$.
[b] Mean rise of temperature in three rabbits upon injection of 50 µgequiv./kg.
[c] Tested in duplicate; relative SEM about 10%.

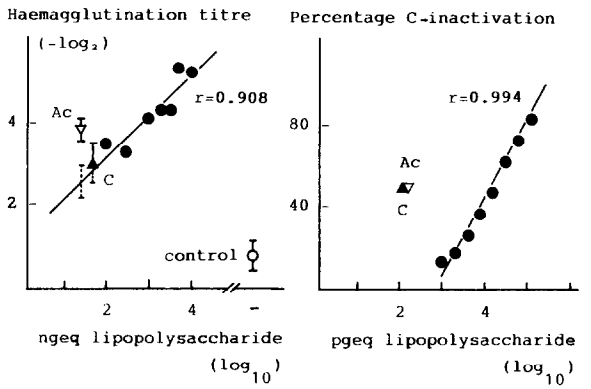

Fig. 2. Dose-effect curve for the adjuvant activity of lipopolysaccharide on the hemagglutinin response of mice ($n=5$) to asialo-SRBC compared to the effectiveness of house dust fractions Ac and C (left panel); idem for complement inactivating capacity (right panel; the mean relative SEM was 10%; $n=3$).

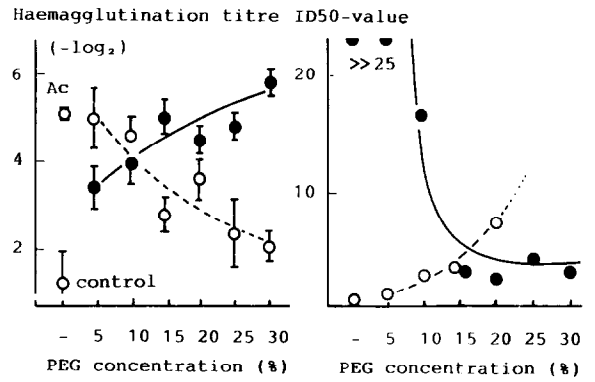

Fig. 3. Adjuvant activities and ID_{50}-values as determined in the precipitates (●) and supernatants (○) resulting from precipitation of fraction Ac with incremental amounts of PEG.

charide. The co-ordinates for fraction C fitted in the regression line. The adjuvant activity of fraction Ac, however, was significantly higher than expected on the basis of its apparent endotoxin content. The plot of doses versus anti-complementary activity yielded an entirely different picture (Fig. 2, right hand panel). Neither the AC nor the C fraction fitted in the dose-response curve for endotoxin. The anti-complementary activities of fractions Ac and C are given in Table 1. Fraction C was twelve times more potent than fraction Ac.

The results suggested that the adjuvant activity of fraction C was due to endotoxin and that fraction Ac probably contained another adjuvant. The crude house dust fraction was therefore subfractionated by PEG precipitation (anion exchange and gel permeation were less successful). The PEG fractions were tested for adjuvant and anti-complementary activities. PEG precipitates of fraction Ac showed adjuvant activity which gradually increased with the PEG concentration (Fig. 3, left hand panel); for the supernatants a proportional decrease in adjuvant activity was observed. Anti-complementary activity, on the other hand, showed a relatively sharp precipitation profile (Fig. 3, right hand panel); the bulk of activity was precipitated between 10 and 20% PEG. Supernatants from solutions with over 20% PEG interfered with the hemolytic complement assay, precluding correct evaluation.

It was further investigated whether the PEG-fraction with the highest adjuvant activity (precipitated at 30% PEG; fraction P) contained endotoxin and whether contamination by this microbial product was responsible for the adjuvant and anti-

Table 2
Data obtained for house dust fraction P, endotoxin and saline.

	Endotoxin content LAL assay (ng/mgequiv.)[a]	Pyrogenicity Δt (°C)[b]	Anti-complementary activity (ID_{50} value; ngequiv.)[c]	Adjuvant activity (per 15 ngequiv. endotoxin)	Complement inactivation (%/150 pgequiv. endotoxin)[e]
Fraction P	48 ± 14	0.73 ± 0.09	2900	2.7 ± 0.3	50
Endotoxin	10^6	n.d.	17	2.4 ± 0.3	0
Saline	-	-	-	0.8 ± 0.3	0

a, b, c For these legends see Table 1.
d Hemagglutination titre ($-\log_2$).
e Relative SEM maximally 10%.

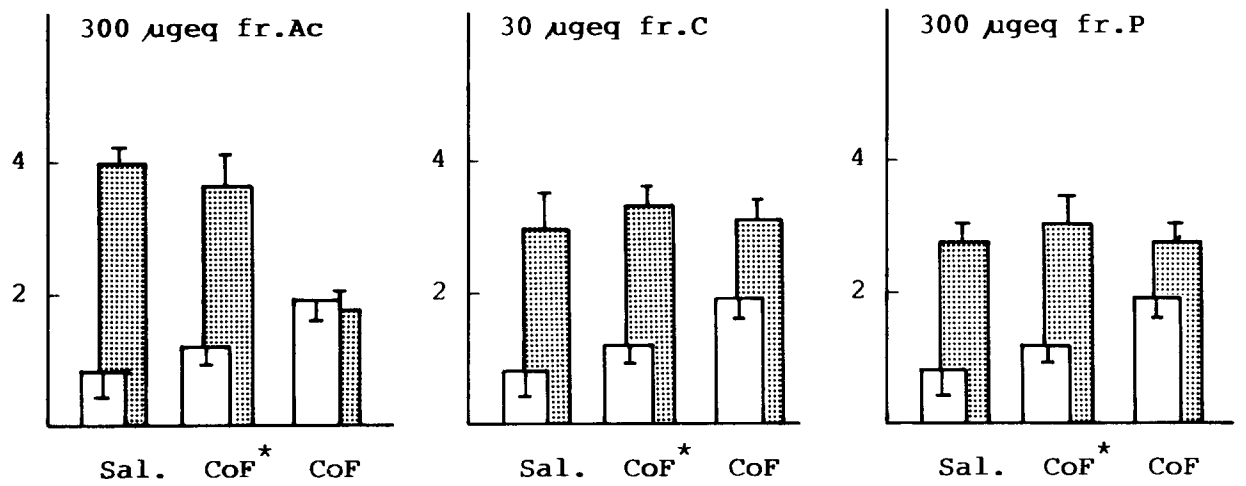

Fig. 4. Effect of CoF treatment of mice on the adjuvant activities of house dust fractions Ac, C, and P for the haemagglutinin response of mice ($n=5$) to asialo-SRBC. Control animals received saline or heat-inactivated CoF (CoF*) instead of active CoF.

complementary activities observed. The apparent endotoxin content and anti-complementary activity of fraction P (Table 2) were of the same order of magnitude as those of fraction Ac (Table 1). With regard to the adjuvant activity, 300 µg of fraction P corresponded to the equivalent amount of commercial lipopolysaccharide (15 ngequiv., Table 2). The anti-complementary activity of fraction P, however, was considerably higher than expected on the basis of its apparent endotoxin content.

In a final experiment, the effect of normal and heat-inactivated CoF on the adjuvant activities of house dust fractions Ac, C, and P was examined. As shown in Fig. 4, CoF suppressed the adjuvant activity of fraction Ac, but not that of the subfractions. In the absence of house dust fractions, CoF by itself exhibited minor, though insignificant, adjuvant activity.

5. Discussion

The adjuvant activity of house dust and the involvement of complement in this property was investigated. A crude house dust fraction (fraction Ac) and two subfractions (fraction C according to Berrens [4] and fraction P prepared by PEG precipitation) showed potent adjuvant activity in vivo compared to pure dextran sulphate, the reference adjuvant in the assay used. Moreover, the house dust (sub)fractions caused (in)activation of mouse hemolytic complement in vitro. Based on the dose–response curve for commercial lipopolysaccharide in the adjuvant assay, the activity of the subfractions could be fully explained by endotoxin, while that of fraction Ac could not. With respect to the anti-complementary activity of the three house dust fractions, endotoxin seemed of minor importance which confirms earlier findings dealing with fraction C [3].

The lack of adjuvant activity of fraction Ac in complement-depleted mice suggests that this fraction contains a complement-dependent adjuvant. So far, the polyanionic adjuvant dextran sulphate and the surface-active compound dimethyldioctadecylammonium bromide have also been shown to act in an apparently complement-dependent way [20]. The adjuvant activity of the two house dust subfractions was obviously complement-independent.

The results of this paper imply that house dust contains at least two immunological adjuvants; one is apparently complement-dependent as shown by its lack of activity in CoF-treated mice, and the other,

probably endotoxin, is not. The initial idea that the most anti-complementary factor and putative adjuvant in fraction C might be identical and involve DNA or DNA-fragments [3] was not sustained. The identity of the complement-dependent adjuvant in fraction Ac remains unclear. It has to be investigated whether this component is also present in samples of other geographic areas.

The complement-activating property of fractions C and P on the one hand, and the apparent complement-independence of their adjuvant activity on the other suggest that complement-activation by an adjuvant is not necessarily involved in its immunostimulatory activity [12–15, 20].

The adjuvants present in house dust may not only be involved in the stimulation of the IgM, but also of the IgE response. Further studies will deal with the effects of the adjuvants in house dust on an experimentally induced IgE response in the mouse and the role of complement in that particular process.

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