

Allergy to guinea pigs: II Identification of specific allergens in guinea pig dust by crossed radio-immunoelectrophoresis and investigation of the possible origin

A. F. WALLS*, A. J. NEWMAN TAYLOR† and JOAN L. LONGBOTTOM

Department of Allergy and Clinical Immunology, and †Department of Occupational Medicine, Cardiothoracic Institute, Brompton Hospital, London, SW3 6HP, U.K.

(Received in original form 1 May 1985 and in revised form 4 July 1985; accepted for publication 11 July 1985)

Summary

An extract of dust from the air-vent filters of a room housing guinea pigs was analysed by quantitative immunoelectrophoretic procedures and compared with extracts of various materials derived from guinea pigs. Crossed radio-immunoelectrophoresis (CRIE) of the dust, performed with sera from twenty asthmatic patients who were positive by skin testing and RAST to guinea pig extracts, identified fourteen IgE-binding constituents. Although responses varied, most sera reacted with four particular allergens, antigens 2, 3, 10 and S1. The numbers of allergens recognized by individual patients correlated with the RAST score, but not with total serum IgE.

All seventeen dust constituents detected by crossed immunoelectrophoresis (and all four major allergens), were also present in extracts of guinea pig dander, fur, saliva and urine; several of these components were absent in an epithelial extract, and there were even less in preparations of shaved pelt, serum or faeces. None of the dust extract antigens were detected in materials used in animal husbandry, dust samples from rooms without guinea pigs, or a *D. pteronyssinus* extract. These findings suggest that inhalant allergens may be derived predominantly from material shed from the guinea pig coat after contamination with saliva, and possibly to a lesser extent, urine.

Introduction

The guinea pig is a most important source of mammalian allergens. It enjoys widespread popularity as a pet, is widely used as a laboratory animal, and several studies have demonstrated that the prevalence of sensitivity to the guinea pig is particularly high.

* Correspondence: Dr A. F. Walls, Department of Biology, University of York, Heslington, York, YO1 5DD.

Fifty-seven of the 945 asthmatic patients who presented at a German allergy clinic were found to keep guinea pigs at home, and of these thirty-five (61%) were skin test positive to a guinea pig hair extract [1]. In another clinic in Germany, Schultze-Werninghaus reported that of 355 out-patients investigated, seventy were exposed to guinea pigs, of whom twenty-seven (39%) were skin test positive and as many as thirty-five (50%) suspected of sensitivity to this species [2].

The guinea pig has been implicated as a more common cause of allergic symptoms than most other animals to which exposure is common. 14.7% of asthmatic patients presenting at a clinic in the Netherlands were skin test positive to guinea pig-derived extracts, and this percentage ranked second only to that for the cat (19.8%) [3]. Similarly, in a large Danish survey, positive skin test reactions to the guinea pig were high in relation to those to many other species [4]. Moreover, Rudolph has reported that the latency period between first exposure and onset of rhinitis or asthma was shorter for the guinea pig than for other common domestic animals, and that the proportion of guinea pig-sensitive patients with asthma was also higher [5].

Recently too, attention has been directed towards the guinea pig as a cause of occupational allergy in laboratory animal handlers, a disease affecting up to 30% of those exposed [6-15]. Most studies of this problem have regarded laboratory animal allergy as a single entity, and have tended not to differentiate between the allergic responses to individual animal species, or to express the prevalence levels to each species in a way which relates the numbers of workers affected to the numbers exposed to each animal species. However, Lutsky & Neuman reported that 24% of the 191 subjects identified as allergic to laboratory animals were sensitive to guinea pigs [11]. Sensitivity to this species was diagnosed also, in at least five of the fifteen animal allergic subjects investigated by Beeson and colleagues [6].

It is clear, therefore, that guinea pig allergens have strong sensitizing properties. Nevertheless, there have been few attempts to investigate the specific allergens involved and there has been an inconsistent approach in the use of material for diagnosis of sensitivity to guinea pigs, with preparations of diverse source materials being employed for this purpose [16].

In view of the lack of information on the sources of guinea pig allergens, the most appropriate reference material is likely to be the dust which accumulates in a room housing guinea pigs, as this may be considered representative of the inhalant material. Moreover, inhibition of the dust RAST with the various guinea pig-derived materials provides a measure of allergenic potency similar to that found by skin tests [16].

The aims of this study were to investigate the antigenic composition of guinea pig dust using quantitative immunoelectrophoretic procedures; to determine the sources of components in various other materials derived from or associated with guinea pigs; and to identify specific guinea pig allergens using crossed radio-immunoelectrophoresis (CRIE).

Materials and methods

Patients

The patients all had rhinitis and asthma, provoked by exposure to guinea pigs and were positive on skin testing and radioallergosorbent test (RAST) to guinea pig-derived extracts.

Collection and preparation of extracts

Source materials were obtained [16] and extracted [17] as described previously. The dust was collected from the ventilation grill of a room housing Dunkin Hartley guinea pigs. Animals were supported on wire mesh floors placed over trays for collection of urine. The trays contained no absorbent material, but were rinsed out regularly. No other animal species were housed on that site.

Male Dunkin Hartley guinea pigs, aged 10–16 weeks, were the source of all guinea pig-derived extracts. Fur was clipped from flanks, epithelial scales were scraped off with a razor blade, and the shaved pelt was also excised. In addition, dander was collected from a sloped stainless steel tray placed under guinea pigs housed individually on a fine wire mesh flooring. The dry skin scales and fur (as well as some crumbs of diet) were collected only from that part of the tray distant from the corner in which urination and defaecation had occurred. Urine was collected with metabolic cages; saliva and faeces were stimulated by subcutaneous injection of pilocarpine nitrate; serum was from blood withdrawn from the heart.

Clean sawdust, hay and diet (SDS Diets, Witham, Essex, U.K.) were collected (though sawdust was not employed as bedding in the room from which the dust sample was obtained).

Extraction, after defatting dried solid source materials in diethyl ether, was in Coca's solution (pH 7.2) for 3 days. These extracts, and liquid source materials were filtered (0.45 μ pore size), dialysed against 0.05 M ammonium hydrogen carbonate (pH 7.8) and freeze-dried.

Antisera

New Zealand White/Dutch Lop crossbred rabbits were immunised with guinea pig-derived extracts in Freund's Complete Adjuvant according to a method described elsewhere [18]. Each bleed was tested individually in crossed immunoelectrophoresis (CIE) and an antiserum pool which allowed optimum resolution of dust constituents produced. This anti-dust pool comprised fifteen bleeds from four rabbits, and included antisera raised against the dander and the urine, as these allowed detection of new dust antigens. Antisera raised against saliva and faeces did not reveal the presence of additional dust constituents, and were not included in the pool.

The anti-guinea pig serum employed, contained ten bleeds from one rabbit immunised with the serum. Anti-rabbit serum, raised in swine, was obtained commercially (Dako Immunoglobulins, Mercia Brocades, West Byfleet, U.K.).

Crossed immunoelectrophoresis (CIE)

CIE was performed essentially as described previously [18, 19], with 1% agarose (BDH, Poole, U.K.) in 0.03 M veronal buffer (pH 8.5) and with 0.0025% thiomersal preservative added. 8.4 $\times$ 8.4 cm glass slides (Photoscience, Canvey Island, U.K.) provided the support for gels.

Ten μ g freeze-dried extract (10 mg/ml in 0.9% NaCl/0.05% NaN₃) was applied to a well and electrophoresed in the first dimension at 4 V/cm, for 60 min. In the second dimension, the separated components were electrophoresed (1.25 V/cm for 20 hr) into an intermediate gel (0.8 $\times$ 5 cm) containing anti-guinea pig serum (12.5 μ l/cm²) and a main gel (3.5 $\times$ 5 cm) containing the pooled anti-dust antisera (22 μ l/cm²). Electrophoresis in the first dimension for 15 min, did not reveal the presence of new faster migrating

antigens; and no cathodal migrating dust components were detected when appropriately placed antiserum containing gels were added.

Tandem CIE [18, 20] was carried out with a second extract added to a well, in line with, and 0.5 cm behind, that which contained the dust extract. Slides were washed overnight with 0.9% saline (containing 0.05% sodium azide), dried and stained with Coomassie brilliant blue stain.

Crossed line immunoelectrophoresis (CLIE)

CLIE [18, 21] was carried out essentially as for CIE except a second intermediate gel (0.8×5 cm) was added below that containing anti-guinea pig serum. This intermediate gel contained a second antigen extract, and where there was cross-reactivity with antigens of the CIE pattern, there was the formation of a line of identity.

Crossed Radioimmunoelectrophoresis (CRIE)

CRIE was based on a modification [22] of the method devised by Weeke & Løwenstein [23, 24]. Wax crayon was applied to the outer extremities of dried CIE slides in order to render them hydrophobic. This allowed the central area alone (5.0×4.5 cm), to be incubated with 4 ml 10% patients' serum in phosphate buffered saline (PBS) (pH 7.2), for 20 hr; and then with 4 ml ^{125}I -anti-IgE (Pharmacia, Uppsala, Sweden) diluted in 0.3% human serum albumin (HSA) in PBS (400 000 cpm per slide) for a further 20 hr.

Before staining, the washed and dried CRIE slides were placed under X-ray film (Kodak NS-2T, Eastman Kodak, Rochester, New York, U.S.A.), initially for 7 days, and then for 2 days, producing two autoradiographs for each slide.

Patients

Patients had been exposed to guinea pigs either at home or in animal houses at various different research institutes. All were asthmatic and were positive by skin-prick testing and RAST to guinea pig extracts.

Skin-prick testing

All patients were skin-prick tested as described previously [17, 22] with extracts of urine from the guinea pig, rat, mouse and rabbit. Concentrations of up to 1 mg/ml extract were employed, and a weal of 3 mm diameter or greater after 20 min was considered as positive.

Radioallergosorbent test (RAST)

RAST for the determination of serum levels of IgE specific for guinea pig dust was carried out as detailed elsewhere [16, 25] with the dust extract coupled to cyanogen bromide activated Sepharose 4B (Pharmacia), and using ^{125}I -anti-IgE (Pharmacia).

PRIST

The PRIST procedure was applied to the measurement of levels of total serum IgE [26] (Phadebas—PRIST[®], Pharmacia).

Results

Antigenic characterization of dust

A reference CIE precipitin pattern was established for the extract of dust collected

from a room housing guinea pigs and the seventeen immunoprecipitates detected were numbered arbitrarily (Fig. 1). Interposing an intermediate gel containing anti-guinea pig serum improved the resolution of peaks and indicated that four components were present in serum (S1, S2, S3 and S4).

Antigen S1 was identified as guinea pig albumin in CLIE, using a commercial preparation of guinea pig serum albumin (Sigma, Poole, U.K.) further purified by Sephacryl S-200 gel filtration chromatography.

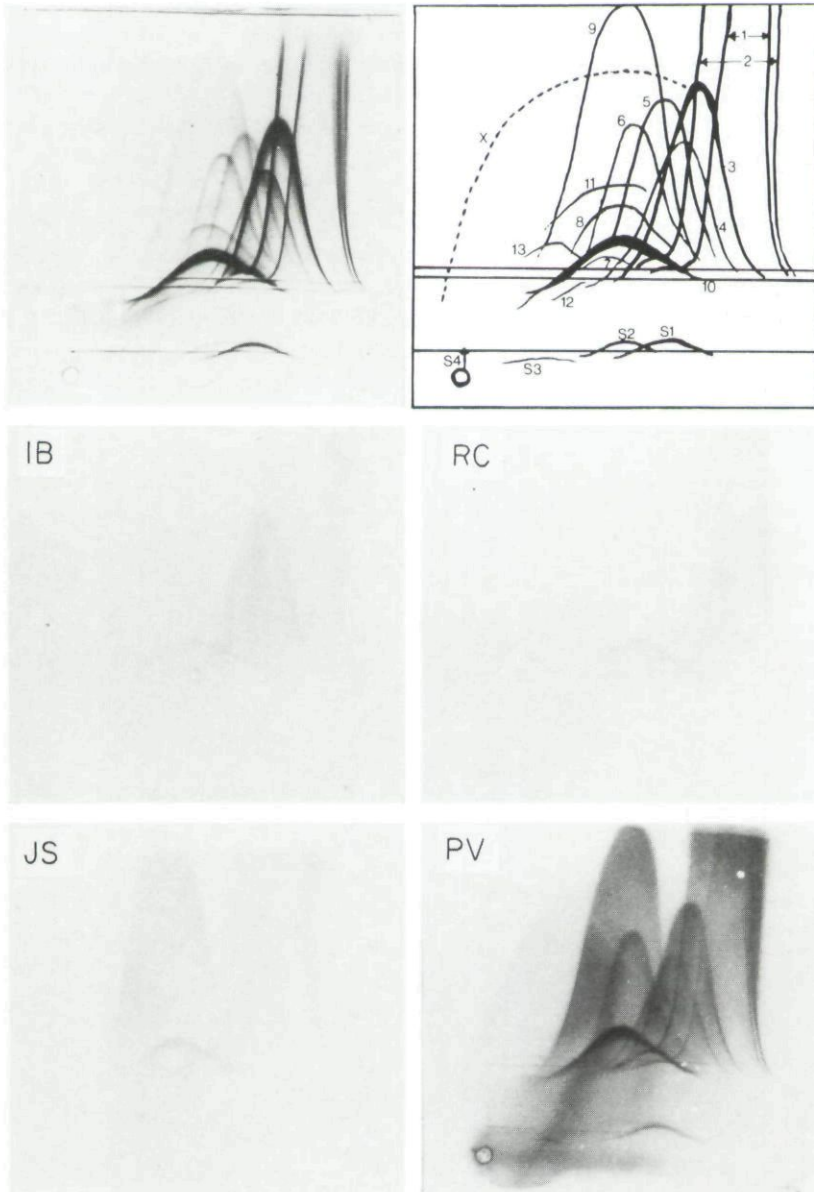


Fig. 1. CIE reference precipitin pattern for guinea pig room dust, with anti-guinea pig serum in the intermediate gel, and anti-guinea pig dust in the main gel (top left). A line drawing indicates the designation of individual antigens (top right); and 7-day CRIE autoradiographs are shown for four patients.

Comparison with other extracts

With 0.5 mg extract in an intermediate gel in CLIE, the extent of antigenic cross-reactivity with each dust antigen was determined.

No identity was observed between any dust antigen and the constituents of extracts of hay, diet, sawdust, housedust, *Dermatophagoides pteronyssinus*, or even dust from a room housing only rats. For the preparations of dander, fur, epithelia, shaved pelt, saliva, urine and faeces, the presence or absence of a reaction of identity with individual dust components is shown in Table 1. In all cases, in the system studied, the reaction was seen as one of complete identity [27].

CIE was performed for dust, dander, fur, epithelia, shaved pelt and serum, using the same anti-dust antiserum pool and the same conditions (Fig. 2). Some of the immunoprecipitates which cross-reacted with specific dust antigens were identified using tandem CIE.

CRIE analysis of dust extract

Autoradiographs for four guinea pig-sensitive patients are illustrated (Fig. 1), and CRIE results tabulated for all 20 patients tested (Fig. 3). A precipitate which was not visible on the stained CIE plate, was observed on CRIE with sera from certain patients (antigen X). Sera from two patients (AB, JP) were tested twice in CRIE of dust and the duplicate autoradiographs were virtually identical. An allergogram indicates the total numbers of patients who reacted with each antigen (Fig. 4).

Table 1. Cross-reaction (+) of guinea pig dust antigens with other guinea pig-derived extracts

Dust Antigen	Dander	Fur	Epithelia	Shaved Pelt	Saliva	Urine	Faeces
1	+	+	+	+	+	+	
2	+	+	+	+	+	+	
3	+	+	+		+	+	
4	+	+	+		+	+	+
5	+	+			+	+	
6	+	+	+		+	+	
7	+	+			+	+	
8	+	+	+		+	+	
9	+	+	+		+	+	
10	+	+	+		+	+	
11	+	+	+		+	+	
12	+	+			+	+	
13	+	+			+	+	
S1	+	+	+	+	+	+	+
S2	+	+	+	+	+	+	+
S3	+	+	+	+	+	+	
S4	+	+	+	+	+	+	+
Total							
17	17	17	13	6	17	17	4

Lack of cross-reactivity in the CLIE system is not shown. There were no missing values.

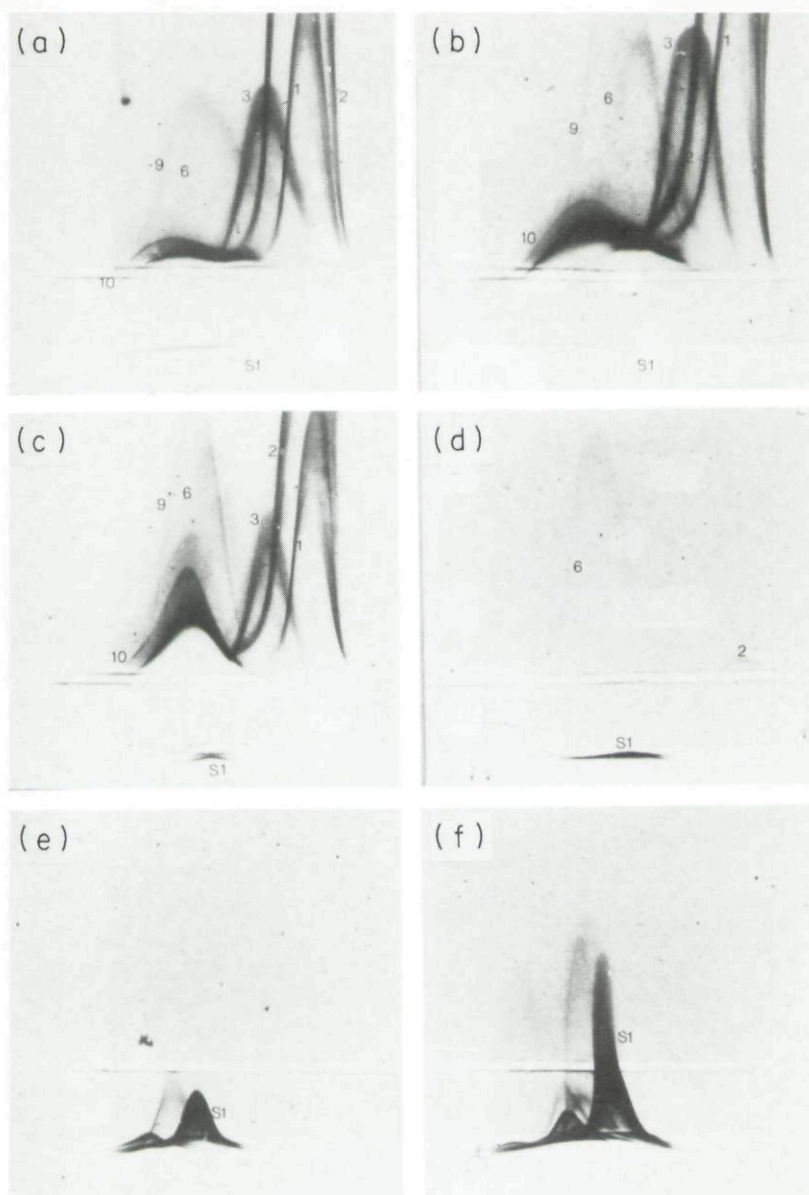


Fig. 2. CIE with 10 μ g of (a) guinea pig room dust; and (b) dander, (c) fur, (d) epithelium, (e) shaved pelt and (f) serum, derived from male guinea pigs. The conditions of CIE, antiserum pools (anti-serum in the intermediate gel; anti-dust in the main gel), and concentrations were the same with each extract.

When CRIE was performed with sera from four asymptomatic subjects, or with a pool of twelve control sera (percent binding in RAST for guinea pig dust, 0.7; total serum IgE, 70 U/ml), or with 0.3% HSA in PBS, in place of serum, there was no reaction except for slight uptake of ^{125} I-anti-IgE on dust antigen 10, with a 7-day incubation. In each case, however, this was less intense than that observed for any of

Patient	Allergy to	RAST % binding	Total IgE (U/ml)	Radiostaining of dust antigen number													
				1	2	3	4	5	6	9	10	12	13	x	S1	S3	S4
P.V.	G	29.9	800	■	■	■	■		■	■	■				■		
R.F.	G(M)	20.1	124	■	■	■	■			■	■				■		
P.E.	(G)RMB	16.1	162		■	■	■				■				■		
A.B.	G(RMR)	15.0	148					■			■				■		
G.S.	GRMB	13.9	70		■	■	■		■	■	■				■		■
P.K.	GRMB	11.8	50		■	■			■	■							
C.S.	(G)R(MB)	10.0	580								■				■		
J.S.	(G)RM(B)	9.3	240		■	■				■	■				■		
J.P.	G	9.2	56		■	■			■		■						
C.A.	(G)R	8.8	570								■						
H.F.	(G)RM(B)	8.6	330								■						
R.C.	G	8.0	41	■	■						■						
R.B.	(G)RM	6.5	530								■				■		
I.B.	G(RM)	6.1	58			■	■										
T.B.	GRM(B)	4.3	62			■	■										
A.C.	GRMB	4.1	104	■	■	■					■						
D.C.	(GRM)B	3.2	570								■						
L.L.	GRM	2.1	490	■	■	■					■				■		
G.H.	GRM	1.8	70							■	■				■		
D.A.	(G)R(MR)	1.1	42	■				■			■						

Fig. 3. CRIE radiostaining of guinea pig dust antigens, with sera from each of twenty patients. A hatched square indicates the presence of a peak on a 2-day autoradiograph, and a dotted square on a 7-day autoradiograph. Also shown is the percent binding in a RAST for IgE to guinea pig dust, the total level of serum IgE (by PRIST), and the presence or absence of a positive skin test reaction to a 1 mg/ml urine extract from the guinea pig (G), rat (R), mouse (M) and rabbit (B). Where the patient did not recognize exposure to a particular species as the cause of symptoms, the letter has been placed in parenthesis.

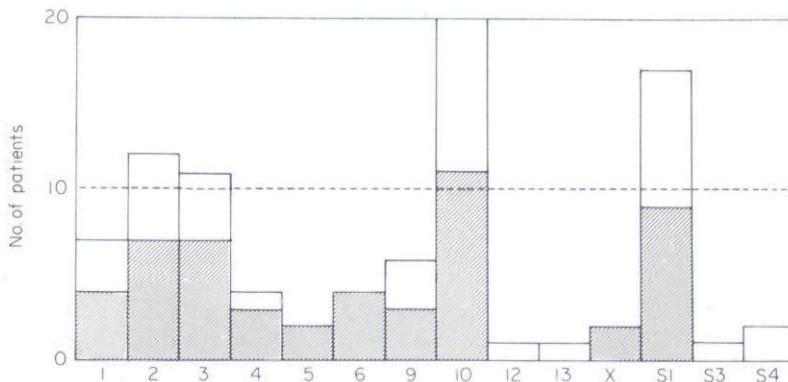


Fig. 4. Allergogram of guinea pig dust, showing the numbers of patients whose serum caused radiostaining of an antigen peak after two days autoradiography (hatched bars), and the numbers after 7 days (open bars).

the allergic sera (results not illustrated). Diffuse radiostaining across the entire gel area, as reported for sensitivity to rabbit serum proteins [28] was not observed for any sera.

By calculation of Spearman's coefficient of rank correlation (r_s) [29], no relationship was found between total IgE of patients' sera (Fig. 3) and the number of antigens radiostained. There was, however, a highly significant correlation between RAST score (Fig. 3), and the number of dust components recognised as allergenic by CRIE ($r_s = 0.527$; $P < 0.001$).

CRIE analysis of rabbit serum

Sera of certain patients (PV, RF, GS) which appeared to react strongly in CRIE with almost every antigen of the guinea pig dust extract, were tested in CRIE for the presence of IgE specific for rabbit serum antigens. The only ^{125}I -anti-IgE uptake observed after 2- and 7-day autoradiography was on the precipitate of rabbit IgG (results not illustrated); but this was of equal intensity for the three allergic sera, for three control sera tested simultaneously, and for a 0.3% HSA in PBS control.

Discussion

The dust from a room housing guinea pigs was used as a basis for determining the source of inhalant allergens for those subjects sensitive to this species. A multiplicity of allergens in the dust extract were identified by CRIE, there being as many as thirteen of the seventeen dust antigens visible on CIE that bound patients' IgE. In addition there was a component visualised only by CRIE. Considerable patient variation also existed in the spectra of antigenic components to which serum IgE was directed.

Several methods have been proposed for classifying allergens on the basis of CRIE experiments. In one scheme, a major allergen has been defined as an antigenic component for which 50% or more patients have IgE, and for which at least 25% show strong specific IgE binding [30–33]. If this arbitrary scheme is applied to the CRIE analysis of guinea pig dust, then four constituents come into the category of major allergen, i.e. antigens 2, 3, 10 and S1.

The roles of these 'major' guinea pig allergens are being studied further [34]. However, some of the radiostaining of antigen S1 (albumin) and to a lesser extent antigen 10, could be due to co-precipitation of other allergens in the immunoprecipitate [35] since the positions of these antigens in the reference pattern render them susceptible to this phenomenon.

Although the importance of allergen co-precipitation is difficult to assess, there was otherwise a high degree of specificity in the CRIE radiostaining, as verified by the relative lack of ^{125}I -anti-IgE uptake with control sera. The little radiostaining which was observed on control autoradiographs was probably due to non-specific binding of ^{125}I -anti-IgE rather than patients' IgE, because it also appeared when HSA in PBS was used instead of patients' serum in CRIE. Moreover, there was no association between numbers of IgE binding antigens and levels of total serum IgE. Intra-assay variation in CRIE was negligible; and there was no evidence for artefactual radiostaining caused by IgE specific for the rabbit antibody, when certain high binding sera were tested in CRIE of rabbit serum.

All of the patients studied had serum IgE specific for several dust components; and the IgE uptake, in terms of the number of allergens recognized, correlated with the RAST score. There were no apparent differences between those patients who attributed their symptoms to guinea pig exposure and those who did not specifically

implicate this species prior to skin testing, either in CRIE responses or in results of RAST or PRIST. As has been discussed elsewhere [22], identification by the patient of the animal provoking symptoms cannot be taken as an infallible guide to sensitivity, where exposure is often to several species on the same site.

The preparation of guinea pig dust was obtained from a site where only guinea pigs were housed. The failure to demonstrate cross-reactivity between guinea pig room dust and a rat room dust extract by CLIE, is in accord with the findings of RAST inhibition [16, 17]; and suggests that there is little antigenic similarity between the inhalant allergens of guinea pigs and rats.

All the dust components observed in the CIE reference pattern appear to be derived from the guinea pigs themselves, rather than from material or organisms associated with them. No reaction of identity was found in CLIE between the guinea pig dust extract and preparations of the diet, hay, sawdust, housedust or *D. pteronyssinus*. Moreover, all seventeen dust antigens seen on CIE, were present in the extracts, of dander, fur, urine and saliva (in which any contamination must have been minimal).

Presumably any non-guinea pig-derived antigens in the dust were present in only trace amounts, or were only weakly immunogenic and did not stimulate antibody production in the rabbits. The effects of random variation in rabbit antibody responses was minimized by selecting an antiserum pool for CIE which comprised several bleeds from four rabbits. Inhalant allergens in the room studied appear therefore to have originated predominantly from the guinea pigs.

The precise source of the dust constituents is more difficult to identify because of the antigenic similarity of the various guinea pig-derived source materials. A cutaneous or faecal origin of the inhalant allergens is however unlikely, since in the extracts of shaved pelt and faeces relatively few dust antigens were detected, and certain of the major allergens were missing.

The antigenic composition of the dust resembled that of the fur, urine and saliva, and also of material shed naturally from the guinea pig's coat (dander). It has been suggested that saliva and urine are the major contaminants of the guinea pig pelt and hence major constituents of dander and therefore that these materials could be the most important sources of the allergens [16]. In this study, both qualitative and quantitative similarities between dust, dander and fur were revealed by CIE using antiserum to the dust (Fig. 2). Epithelial scrapings and, to a much lesser extent, shaved pelt contained some of the antigens prominent in the dust, but in relatively smaller amounts, whilst layers of the pelt closer to the skin were found to contain progressively more serum protein (and this has been found by skin testing [6, 16, 36], and RAST inhibition [16], to be of low allergenic activity).

Saliva may represent a more important source of guinea pig allergens on fur than the urine. Self-grooming occurs frequently [37] and relatively large amounts of salivary protein can be excreted [34]. Allergens from saliva will be applied directly, and in high concentrations to the coat, whereas contamination of fur with urine will be indirect and incidental and is seldom evident [38].

Urinary allergens could also become airborne if allowed to dry onto light particulate bedding materials; but this was unlikely to have been a major source of inhalant allergens in the room from which the dust sample was collected, since the animals were housed in cages with wire mesh floors, and the trays beneath (in which there was no absorbent material) were regularly drained and rinsed out. Although hay was added to cages as bedding and as a supplement to the diet, the tendency of guinea

pigs to restrict urination to the corners of cages is not likely to result in appreciable contamination. There would thus appear to have been relatively little opportunity for urine contaminated bedding to become airborne (and neither hay nor diet cross-reacted with the dust extract.)

The relative importance of different sources of allergens may be affected by the method of husbandry and conditions of exposure to the guinea pig. Nevertheless, when guinea pig behaviour is considered in conjunction with the dust analysis of this study, it would seem that in many cases, salivary proteins shed in dander could represent the most important source of inhalant allergens.

Acknowledgments

We gratefully acknowledge advice and assistance from Dr M.W. Elves and Dr D. Turner, and clinical help from Dr M. Dally, Miss Rosemarie Hawkins, Dr R.E.C. Altounyan, Dr A.J.M. Slovak, Dr C.A.C. Pickering, Dr S.R. Durham and Professor A.B. Kay. Thanks are due also to Miss Alexandra Tee for typing, to Mrs Rosemary Kay for artwork and to the Photographic Service of the Royal Marsden Hospital.

This work was supported by the Science and Engineering Research Council and Glaxo Group Research plc.

References

- 1 Bruchhausen D, Bruchhausen M. Beobachtungen über Meerschweinchen- und Goldhamster-haarallergien. *Z Allgemed* 1975; 51:1600-2.
- 2 Schultze-Werninghaus G. Atemweg serkrankungen durch soforttypallergie gegen tierepithelien. *Allergologie*, 1981; 4:298-303.
- 3 Muljono IS, Voorhorst AB. Atopy from dander to domestic animals. *Allergol Immunol* 1978; 24:50-60.
- 4 Østerballe O, Dirksen A, Weeke B, Weeke ER. Forekomst af medicinsk-allergiske sygdomme i speciallaegeregiet. Hvilke allergenekstrakter skal anvendes til priktest ved allergidiagnostik—en dansk multicenterundersøgelse. *Ugeskr Laegr* 1981; 143:3211-18.
- 5 Rudolph R. Frequency of animal sensitisation. In: Kerr JW, ed. *Proceedings of the XIth international congress of allergology and clinical immunology*, London. London: MacMillan Press, 1983: 437-44.
- 6 Beeson MF, Dewdney JM, Edwards RG, Lee D, Orr RG. Prevalence and diagnosis of laboratory animal allergy. *Clin Allergy* 1983; 13:433-42.
- 7 Cockcroft A, Edwards J, McCarthy P, Anderson N. Allergy in laboratory animal workers. *Lancet* 1981; i:827-30.
- 8 Davies GE, McArdle LA. Allergy to laboratory animals: A survey by questionnaire. *Int Archs Allergy Appl Immunol* 1981; 64:302-7.
- 9 Gross NJ. Allergy to laboratory animals: epidemiologic, clinical and physiologic aspects, and a trial of cromolyn in its management. *J Allergy Clin Immunol* 1980; 66:158-65.
- 10 Lincoln TA, Bolton NE, Garrett AS. Occupational allergy to animal dander and sera. *J Occup Med* 1974; 16:465-9.
- 11 Lutsky IK, Neuman I. Laboratory animal dander allergy. I. An occupational disease. *Ann Allergy* 1975; 35:201-5.
- 12 Newman Taylor AJ, Myers JR, Longbottom JL, Spackman D, Slovak AJM. Immunological differences between asthma and other allergic reactions in laboratory animal workers. *Thorax*, 1981; 36:229 (abstr).
- 13 Philips JJ, Lee PB, Williams DI. An initial survey of allergies amongst animal house technicians. *J Inst Animal Technol* 1977; 28:109-11.
- 14 Slovak AJM, Hill RN. Laboratory animal allergy: a clinical survey of an exposed population. *Br J Ind Med* 1981; 38:38-41.
- 15 Taylor G, Davies GE, Altounyan REC *et al.* Allergic reactions to laboratory animals. *Nature* 1976; 260:280.
- 16 Walls AF, Newman Taylor AJ, Longbottom JL. Allergy to guinea pigs: I. Allergenic activities of extracts derived from the pelt, saliva, urine and other sources. *Clin Allergy* 1985; 15: 241-51.
- 17 Walls AF, Longbottom JL. Allergy to rats: a comparison of allergenic potencies for a range of source materials by skin prick testing RAST and RAST inhibition. *J Allergy Clin Immunol* 1985; 75:242-51.

- 18 Walls AF, Longbottom JL. Quantitative immunoelectrophoretic analysis of rat allergen extracts. I. Antigenic characterisation of fur, urine, saliva and other rat-derived materials. *Allergy*, 1983; 38:419-31.
- 19 Longbottom JL, Brighton WD, Edge G, Pepys J. Antibodies mediating type I skin test reactions to polysaccharide and protein antigens of *Candida albicans*. *Clin Allergy* 1976; 6:41-9.
- 20 Krøll J. Tandem crossed immunoelectrophoresis. In: Axelsen NH, Krøll J, Weeke B, eds. *A Manual of Quantitative Immunoelectrophoresis. Methods and Applications*. Scand J Immunol 1973; 2 (suppl. 1):57-9.
- 21 Krøll J. Crossed line immunoelectrophoresis. In: Axelsen NH, Krøll J, Weeke B, eds. *A manual of quantitative immunoelectrophoresis. Methods and applications*. Scand J Immunol 1973; 2 (suppl. 1):79-81.
- 22 Walls AF, Longbottom JL. Quantitative immunoelectrophoretic analysis of rat allergen extracts. II. Fur, urine and saliva studied by crossed radio-immunoelectrophoresis. *Allergy*, 1983; 38:501-12.
- 23 Lowenstein H. Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. *Prog Allergy* 1978; 25:1-62.
- 24 Weeke B, Lowenstein H. Allergens identified in crossed radioimmunoelectrophoresis. In: Axelsen NH, Krøll J, Weeke B, eds. *A manual of quantitative immunoelectrophoresis. Methods and applications*. Scand J Immunol 1973; 2 (suppl. 1):149-53.
- 25 Gad El Rab MO, Kay AB. Widespread IgE-mediated hypersensitivity in the Sudan to the 'green nimitti' midge *Cladotanytarsus lewisi* (Diptera: Chironomidae). I. Diagnosed by radioallergosorbent test (RAST). *J Allergy Clin Immunol* 1980; 66:190-7.
- 26 Ceska M, Lundkvist U. A new simple radioimmunoassay method for the determination of IgE. *Immunochemistry* 1972; 9:1021-30.
- 27 Axelsen NH, Bock E, Krøll J. Comparison of antigens: the reaction of 'identity'. In: Axelsen NH, Krøll J, Weeke B, eds. *A manual of quantitative immunoelectrophoresis. Methods and applications*. Scand J Immunol 1973; 2:(suppl. 1):91-4.
- 28 Aukrust L, Almeland TL, Aas K. The specificity of radiostaining in crossed radioimmunoelectrophoresis. *Scand J Immunol* 1978; 8:421-7.
- 29 Siegel S. *Nonparametric statistics for the behavioural sciences*. Kogakusha, Tokyo: McGraw-Hill, 1956.
- 30 Aukrust L. Allergens in *Cladosporium herbarum*. In: Oehling A, ed. *Advances in allergology*. New York: Pergamon Press, 1980:475-80.
- 31 Aukrust L, Borch M. Purification of allergens in *Cladosporium*. *Allergy*, 1980; 35:206-7.
- 32 Lind P, Lowenstein H. Identification of allergens in *Dermatophagoides pteronyssinus* mite body extract by crossed radioimmunoelectrophoresis with two different rabbit antibody pools. *Scand J Immunol* 1983; 17:263-73.
- 33 Lowenstein H. Crossed radioimmunoelectrophoresis. In: Oehling A, ed. *Advances in allergology and clinical immunology*. Oxford: Pergamon Press, 1980: 573-7.
- 34 Walls AF *et al.* Allergy to guinea pigs: III. Antigenic analysis and comparison of the dander, saliva and urine (in preparation).
- 35 Aukrust L, Grimmer O, Aas K. Demonstration of distinct allergens by means of immunological methods. Comparison of crossed radioimmunoelectrophoresis (CRIE), radioallergosorbent test (RAST) and *in vivo* passive transfer test (PK test). *Int Arch Allergy Appl Immunol* 1978; 57:183-92.
- 36 Ohman JL, Lowell FC, Bloch KJ. Allergens of mammalian origin. II. Characterisation of allergens extracted from rat, mouse, guinea pig and rabbit pelts. *J Allergy Clin Immunol* 1975; 55:16-24.
- 37 Harper LV. Behavior. In: Wagner JE, Manning PJ, eds. *The biology of the guinea pig*. New York: Academic Press, 1976: 31-51.
- 38 Paterson JS. The guinea-pig or cavy. In: *The UFAW handbook on the care and management of laboratory animals*, 4th Edn. Edinburgh and London: Churchill Livingstone, 1972: 223-41.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.