

Isotype specific immunoglobulin responses to the house dust mite *Dermatophagoides pteronyssinus* and the purified allergen *Der p 1* in asthmatic and control subjects from the Eastern Highlands of Papua New Guinea

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Summary

The IgG, IgE and IgA antibody responses to the whole mite extract and a purified major mite allergen *Der p 1*, in sera from asthmatic and age- and sex-matched control subjects from the South Fore region of the Eastern Highlands of Papua New Guinea, have been studied. Radio-allergosorbent studies showed that the majority of the asthmatics, in contrast to control subjects, produced IgE to whole mite extract, and that *Der p 1* was a major allergen in this population with 88% of mite allergic asthmatics responding. Enzyme-linked immunosorbent assay studies on these sera showed that the geometric mean levels of whole mite, but not *Der p 1*, specific IgG and IgA were significantly higher in the asthmatic group than in the control group. Significant correlations between whole mite specific IgG, IgE and IgA responses were obtained. These data indicate that Papua New Guinean asthmatics are similar to Caucasian asthmatic population with regard to serological responses to mite allergens, despite differences in disease presentation, particularly the late age of onset and severity of symptoms.

Introduction

The house dust mite *Dermatophagoides pteronyssinus* has been shown to play an important role in triggering allergic diseases, such as asthma, in many countries throughout the world, both temperate and tropical. Three interesting features of

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asthma in the South Fore region of the Eastern Highlands in Papua New Guinea [1–3] distinguish it from that seen in temperate regions. (i) The frequency of the disease appears to be increasing dramatically, since the prevalence has risen 39–46-fold during the decade 1972–1982 [4,5]. (ii) It is primarily confined to adults, with a mean age of onset for males and females of 32·5 and 24·4 years, respectively [5]. (iii) It is clearly associated with allergy to house dust mite. In contrast, asthma appears early in childhood in temperate climates where it is generally associated with an allergic stimulus, whilst adult asthma has a large intrinsic component [6].

In this report, we have studied the specific immunoglobulin responses to both the house dust mite and a major purified mite allergen *Der p 1* (analogous to Dpt 12, and Dp 42) in asthmatic and control patients, from the South Fore region of Papua New Guinea, to determine whether the serological features of the disease resemble those described in other studies on more typical allergic asthmatic populations.

Subjects and methods

Subjects

Detailed physiological and partial serological studies relating to the asthmatic and age- and sex-matched controls resident in eight South Fore villages of the Eastern Highlands of Papua New Guinea are reported elsewhere, as are the criteria employed to define asthma [4,5].

Preparation of extracts

Dermatophagoides pteronyssinus. The house dust mite *Dermatophagoides pteronyssinus* (free from growth medium), and spent growth medium (free of whole mites) were kindly provided by Commonwealth Serum Laboratories, (Parkville, Australia). A soluble extract was prepared by grinding the mites at 0°C in 0·05 M ammonium bicarbonate, pH 8·0, followed by centrifugation at 15 000 × *g* for 15 min. The supernatant was removed and the insoluble material re-extracted twice more in a similar manner. The supernatants were combined and dialysed exhaustively against 0·05 M ammonium carbonate, pH 8·0, lyophilized and stored dessicated at –20°C until required. Spent growth medium was extracted in a similar fashion.

Preparation of Der p 1. The allergen *Der p 1* was isolated by chromatofocusing from spent growth medium as described earlier [7,8]. Those fractions containing the allergen (pI 6·9–5·8) were pooled, dialysed against 0·05 M ammonium bicarbonate, pH 8·0, and lyophilized. The allergen preparation was finally purified by gel filtration on a P-60 column (Bio Rad Laboratories Pty Ltd, Sydney, Australia) equilibrated with 0·05 M ammonium bicarbonate, pH 8·0. The fractions containing the allergen were pooled and lyophilized.

Allergen specific IgE. IgE antibodies with specificity for whole mite or *Der p 1* were assayed as previously described [5], employing CNBr-activated paper discs coated with either extract and ¹²⁵I-labelled anti-human IgE (Pharmacia South Seas Pty Ltd, Sydney, Australia). Radio-allergosorbent test scores to whole mite extract were expressed as units per ml (U/ml) determined by reference to a standard curve constructed using a mite-allergic serum pool that had been arbitrarily assigned a unit value. Test sera were diluted so that the response would fall on the linear portion of the standard curve, and the resultant unit values were then corrected for dilution. Purified

Der p 1 was optimally coupled to CNBr-activated paper discs, as described previously [7], and RAST scores were expressed as percentage counts bound.

Allergen specific IgG and IgA. IgG and IgA antibody responses to both whole mite extract and *Der p 1* were determined using the enzyme-linked immunosorbent assay (ELISA), essentially as described previously [9]. Antibody titres to whole mite were assayed using a double antibody system employing commercial rabbit anti-human IgG and IgA (Behring, FRG), and alkaline phosphatase (Sigma Chemical Co. Ltd, St Louis, MO, U.S.A.) conjugated immunopurified sheep anti-rabbit IgG, prepared in our laboratory and absorbed with glutaraldehyde insolubilized normal human serum [10]. Enzyme-linked immunosorbent assay plates (EIA plates, Flow Laboratories, U.S.A.) were optimally coated with whole mite extract at a concentration of 50 µg dry weight per ml alkaline carbonate buffer. Responses to *Der p 1* were determined using a single antibody ELISA system with alkaline phosphatase conjugated sheep anti-human IgG Fc fragment or sheep anti-human IgA, and ELISA plates coated with allergen at a concentration of 1 µg/ml. For the whole mite specific IgG ELISA, each serum was diluted 1 in 1000 with 0.01 M phosphate buffer, pH 7.2, containing 0.05% v/v Tween 20 and 0.15 M NaCl (PBST), whereas for the whole mite specific IgA ELISA each serum was diluted 1 in 84. For both the *Der p 1* specific IgG and IgA ELISA each serum was diluted 1 in 100 with PBST. The sheep anti-human Fc antiserum was prepared against the Fc fragment obtained by papain digestion of pooled normal human IgG, and absorbed extensively with Fab fragment coupled to CNBr-activated Sepharose 4B. The sheep anti-human IgA antiserum was raised against a human myeloma IgA purified by hydrophobic interaction chromatography [11], and the resultant antiserum absorbed with glutaraldehyde insolubilized cord serum. The specificity of the antisera were checked using plates coated (10 µg/ml) with F(ab)₂ fragment, IgA and IgG. A positive and negative mite-allergic serum control was included on each plate in at least triplicate, and the values from different plates were normalized. The data were expressed as optical densities at 405 nm (OD), and were statistically evaluated using the statistical package of the Western Michigan University, U.S.A.

Skin testing

Skin-prick tests were performed on the flexor aspect of the forearm using *D. pteronyssinus* extract, (Hollister-Stier, U.S.A.), solvent controls and histamine (1 mg/ml) as described [5]. The minor and major axes of the main body of the weals were measured (in mm) 15 min after challenge, and the mean values taken. Reactions with a mean diameter of 2 mm, and greater, after subtraction of the solvent control, were judged to be significant. The *Der p 1* content of the *D. pteronyssinus* skin-test extract used in this study was not determined, but studies performed on similar extracts from this manufacturer [12] have shown that they contain approximately 35 µg of *Der p 1* per ml extract.

Results

Mite specific IgE

The levels of both whole mite and *Der p 1* specific IgE in the sera of the adult asthmatic and control subjects are shown in Figs 1 and 2, and Table 1. There was a significantly higher geometric mean level of both whole mite and *Der p 1* specific IgE in the

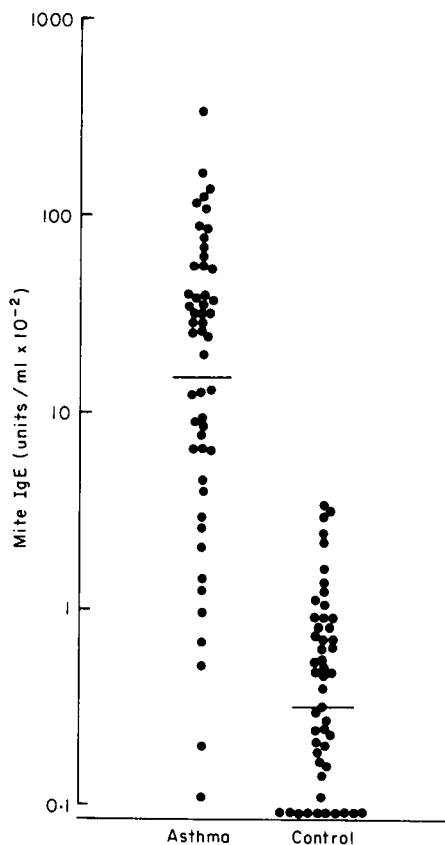


Fig. 1. Distribution and geometric mean (—) of *Dermatophagoides pteronyssinus* specific IgE for the asthmatic and control adult study groups from Papua New Guinea.

asthmatic adults than in the adult controls. The geometric mean level of whole mite specific IgE in serum from asthmatics and control subjects were 1517, and 32 U/ml, respectively. The geometric mean per cent radiation-bound levels in the adult asthmatic patients and adult control subjects were 8.8 and 1.7, respectively (Fig. 2, Table 1).

The frequency with which mite-allergic asthmatic subjects, as defined by positive whole mite skin tests, produced IgE to *Der p* 1 was determined. Of the forty-two (82%) subjects that were skin-test positive (Table 2), thirty-seven (88%) were positive by *Der p* 1 RAST (> 3% radiation-bound) and five were negative (12%). Nine subjects were skin-test negative to whole mite, three were positive by *Der p* 1 RAST. Of these three subjects, the whole mite RAST scores were 850, 762 and 907 U/ml, respectively, and the geometric mean score of this group was significantly higher ($P < 0.001$) than that of the control group (i.e. > 172 U/ml) and, individually, these sera were judged mite allergic. The remaining six subjects who were skin-test negative for whole mite were also *Der p* 1 RAST negative. However, of these six subjects, one was judged mite allergic on the basis of whole mite RAST, and two were judged possible mite allergic.

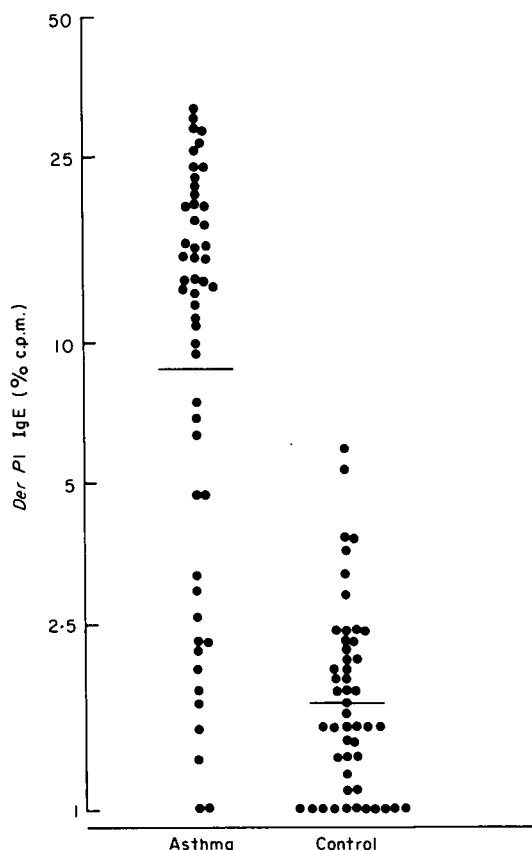


Fig. 2. Distribution and geometric mean (—) of *Der p 1* specific IgE for the asthmatic and control adult study groups from Papua New Guinea.

Whole mite specific IgG and IgA

The geometric mean ODs of whole mite specific IgG and IgA in sera from the asthmatic group were significantly higher than the corresponding values obtained with sera from the control group (Table 1).

Der p 1 specific IgG and IgA

Der p 1 specific IgG and IgA responses were also determined by ELISA. However, it was not possible to differentiate between sera from adult asthmatic and control patients (Table 1).

Correlations between serological parameters

The correlation between various serological parameters in the combined groups was determined by linear regression analysis (Table 3). These studies showed significant correlations between whole mite specific IgE and both whole mite specific IgG and IgA levels, and between whole mite specific IgE and *Der p 1* specific IgE. Correlations between *Der p 1* specific IgE scores and *Der p 1* IgG, and between specific *Der p 1* IgG and *Der p 1* IgA levels were not observed, but a weak correlation between *Der p 1* IgE scores and *Der p 1* specific IgA level was detected ($P < 0.05$).

Table 1. Specific IgE, IgG and IgA responses to *Dermatophagoides pteronyssinus* and the purified allergen *Der p 1* in Papua New Guinean adult asthmatic and control subjects

	Asthma	Control	Probability*
<i>D. pteronyssinus</i> IgE (U/ml)	1517 (11-33575)†	32 (0-342)	$P < 0.001$
<i>Der p 1</i> IgE (% radiation bound)	8.8 (1.3-31.1)	1.7 (0.7-5.9)	$P < 0.001$
<i>D. pteronyssinus</i> IgG (OD 405 nm)	0.26 (0.065-0.78)	0.16 (0.06-0.705)	$P < 0.001$
<i>D. pteronyssinus</i> IgA (OD 405 nm)	0.53 (0.22-1.54)	0.35 (0.16-1.07)	$P < 0.001$
<i>Der p 1</i> IgG (OD 405 nm)	0.27 (0.08-1.22)	0.23 (0.08-1.33)	n.s.
<i>Der p 1</i> IgA (OD 405 nm)	0.14 (0.05-0.72)	0.12 (0.06-0.32)	n.s.

* The significance of the differences between the geometric means of various parameters obtained with the asthmatic and control adults was determined by Student's *t*-test. n.s. = Not significant.

† Figures in parentheses indicate the range of values detected.

Table 2. Correlation between *Der p 1* RAST and skin test reactions to whole *Dermatophagoides pteronyssinus* extract in asthmatic patients from Papua New Guinea

Whole mite skin test	<i>Der p 1</i> IgE*		Total no. subjects
	Positive	Negative	
Positive†	37 (88)‡	5 (12)	42
Negative	3 § (33)	6 (67)	9

* Subjects were judged to be allergic if the *Der p 1* RAST scores were equal to, or greater than, 3% radiation bound.

† Subjects were judged to be mite skin-test positive if the weal diameter was equal to, or greater than, 2 mm after subtraction of diluent control.

‡ Figures in parentheses indicate the percentage of subjects responding.

§ These three patients were judged to be mite allergic on the basis of mite RAST scores.

Similar findings to those described in the above table were obtained if whole mite RAST scores were used to define the mite allergic status of the PNG asthmatics (data not shown).

Table 3. The relationship between various immune responses to whole mite extract and *Der p 1* in asthmatic and control adults from Papua New Guinea

Relationship	Correlation coefficient*	Probability
<i>Whole mite</i>		
IgE vs IgG	0.32	$P < 0.001^{**}$
IgE vs IgA	0.50	$P < 0.001$
IgG vs IgA	0.33	$P < 0.001^{**}$
IgE vs <i>Der p 1</i> IgE	0.77	$P < 0.001$
<i>Der p 1</i>		
IgE vs IgG	0.16	n.s.
IgE vs IgA	0.22	$P < 0.05^{**}$
IgG vs IgA	0.00	n.s.

* The correlation coefficient were determined using log transformed values.

† The correlation coefficients were not significant when only data from the asthmatic group were analysed.

n.s. = Not significant.

Discussion

The studies described here, together with previous studies on the people from Papua New Guinea, have clearly shown an association between allergy to the house dust mite *D. pteronyssinus* and asthma, since significant differences in the amounts of mite specific serum IgE in the asthmatic and control subjects were demonstrated by both skin testing and by RAST [1-5].

In this study, we have shown that the allergen *Der p 1* was a major allergen in the South Fore mite-allergic asthmatic subjects, in that 88% of those who were judged mite allergic either by skin testing and/or RAST had *Der p 1* specific IgE antibodies. The frequency of reactivity is slightly lower than that obtained previously (100%) from this laboratory by RAST using sera from Caucasian children selected on the basis of positive mite RAST scores [7], but similar (79%) to that obtained by Chapman and Platts-Mills [13] using a double antibody assay, and to that obtained (90%) by Krillis *et al.* [14] using crossed radio-immuno-electrophoresis (on the assumption that the allergen designated no. 1 in their study is analogous to *Der p 1*). A good correlation between IgE responses to whole mite extract and *Der p 1* was obtained in accord with previous studies [7,13].

On the basis of these and other data, the observed increase in asthma and its association with allergy to the mite has been ascribed to the recent acquisition of cotton blankets by the South Fore people, which have provided a fertile environment for mite reproduction. In support of this conclusion were the observations that the introduction of blankets preceded the increased prevalence, and that mite numbers in such blankets were significantly higher than those found in other parts of traditional houses [5]. Such conclusions are not without support from other studies in both temperate and tropical climates. For example, it has been shown that all types of bedding, such as mattresses, pillows and blankets, harbour high mite densities [15-18]. The observations that asthmatic patients from the South Fore produce IgE antibodies not only to the whole mite extract but also to *Der p 1* is also consistent with the above conclusions, since the

number of mites correlate with the amount of *Der p 1* in dust samples, and the most potent source of this allergen is bed dust [19].

Enzyme-linked immunosorbent assay studies showed that the geometric mean response of both mite specific IgG and IgA were higher in the asthmatic group than in the control group and these findings are, therefore, similar to those reported for mite-allergic asthmatics and control patients from developed countries [20–24]. Linear regression analysis showed significant correlations between whole mite specific IgE and both mite specific IgG and IgA when the combined populations were examined. However, the only correlations that persisted when the asthmatic population was examined *per se* was that between whole mite specific IgE and IgA, and that between whole mite specific IgE and *Der p 1* specific IgE.

In contrast to the above findings with regard to whole mite extract, it was not possible to differentiate between the South Fore asthmatic and control patients on the basis of their IgG and IgA responses to *Der p 1*. In previous studies, clear differences in serum IgG and IgA responses between mite-allergic and non-allergic patients were observed using a double antibody radioimmunoassay [13]. For example, it was shown that 94% and 65% of mite-allergic patients produced IgG and IgA antibodies to *Der p 1*, respectively, whereas in control subjects, only 30% produced IgG antibodies, and IgA responses were not detected at all. The reasons for this discrepancy are not yet known but it is possible that the *Der p 1* ELISA may be less sensitive than double antibody ELISA systems and radioimmunoassay, or subject to non-specific immunoglobulin binding [25]. However, the observation that the ELISA responses can be inhibited by free allergen (data not shown) suggests that the latter would only be a partial explanation. We are in the process of comparing the ELISA with double antibody assay for the measurement of immunoglobulin responses to *Der p 1*.

In conclusion, we have shown asthmatics from Papua New Guinea, like their Caucasian counterparts, are immunologically hyperresponsive to whole mite allergen in that their serum levels of IgE, IgG and IgA are significantly higher than those found in non-asthmatic controls. In addition, the allergen *Der p 1* is a major (but not necessarily the only) allergen in this population. These data support the previously described association between mite allergy and asthma, and indicate that, in general, the serological features of the Papua New Guinean asthmatics are similar to those described previously for other allergic asthmatic groups, despite the much later age of onset.

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