The house dust mite *Dermatophagoides pteronyssinus* is the most important allergen on the island of Mauritius

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Summary

To determine the relative importance of mites as a cause of allergic sensitivity and asthma on the western Indian Ocean island of Mauritius, we measured specific IgE antibodies to common inhalant allergens in sera from Mauritians claiming to have allergic symptoms and we examined house dust samples for evidence of mites and their allergens. Seventy-two of the 110 sera tested (65%) contained detectable IgE antibody to at least one mite, mould or pollen allergenic extract. By far the most prevalent was antibody to one or both of the common house dust mites, Dermatophagoides pteronyssinus and Dermatophagoides farinae, being present in 67 (61%) of the 110 sera. Allergy to pollens, including the locally prevalent Bermuda grass and sugar cane, was infrequent. Antibody to a limited number of moulds was detected in 22% of the sera tested. Of 81 subjects whose clinical history was known, 60 were asthmatic, and 75% of these asthmatic individuals had IgE antibody to mites. In contrast, only 35% of the subjects with rhinitis without asthma were sensitive to mites. Different mite species, including D. pteronyssinus but not D. farinae, were identified microscopically in samples of local house dust. Mite antigen Der p I but not antigen Der f I was detected with specific monoclonal antibodies in extracts of these dust samples. On the bases of this serological and environmental survey, we conclude that our data support the hypothesis that the house dust mite D. pteronyssinus is the principal cause of allergic sensitivity and asthma in that tropical environment.

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Introduction

Morbidity due to asthma is increasing in the industriallydeveloped temperate parts of the world [1-5]. There is strong circumstantial evidence that the increasing prevalence of asthma in developed countries is related causally to increasing exposure to house dust mites [6-8]. This latter phenomenon appears to be related to changes in living quarters that have occurred in recent years, including tighter insulation, higher indoor temperatures and relative humidity, and the more widespread use of permanently installed wall-to-wall carpets, all changes that favour the growth of house dust mites [9]. Moreover,

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as revealed in the studies of Turner and his colleagues, the same phenomenon has occurred in the tropical environment of Papua New Guinea, where the prevalence of asthma in adults rose from 0.1% to 7.3% in the South Fore region of that island following the introduction of the use of blankets and, consequently, increased exposure to dust mites [10–12]. Mites are also an important cause of allergy in other tropical and subtropical environments [13–15].

In a recent study of the prevalence of allergic disease in a group of 2500 school children on Mauritius, a small island located in the tropical zone of the western Indian Ocean, we found that the prevalence of asthma was 3.5%in that sample of the local population (unpublished data). In light of the now widely accepted correlation between asthma and exposure to house dust mites, as well as the reported correlation between mite densities in the local environment of asthmatic patients and their serum IgE anti-mite antibody levels [6–8,16], we investigated the prevalence of sensitivity to mites in a group of adolescent and adult Mauritians who claimed to have an allergic disease by measuring IgE antibodies in their serum. We also looked for evidence of mites in dust samples from their homes, identifying the species of mites found in the samples and assaying two major mite allergens (*Der p* I from *D. pteronyssinus* and *Def f* I from *D. farinae*) in extracts of these dust samples. In addition, we assayed the sera for IgE antibodies to a wide variety of common inhalant allergens to determine their relative importance viz-a-viz mite allergens.

Materials and methods

Study population

One-hundred-and-ten adolescents and adults claiming to have an allergic disease volunteered to participate in this study. The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983. Although the subjects were recruited in various schools and in the Sir Seewoosagur Ramgoolam (SSR) Centre for Medical Studies in the vicinity of Port-Louis, the capital of Mauritius, they were, in fact, from diverse parts of this small island. With one exception, they had spent most if not all their life on the island. A brief allergic history and a blood sample were obtained from each of them. Although only 81 of the history forms were later exploitable, the general characteristics of the remaining 29 subjects did not appear to be different from those in the larger group. Of the 81 history forms analysed, 39 were from females, ,42 from males. Their average age was 23.7 years (range, 7 to 65 years). Sixty of them were diagnosed as having asthma (43 of whom also had allergic rhinitis), 17 had allergic rhinitis without asthma and four apparently had neither asthma nor rhinitis.

Dust collection and extraction

Bedroom dust (from the floor and mattress) was collected with a 700 W vacuum cleaner at a rate of 2 min/m^2 in homes of asthmatics who lived in various parts of the island. The dust was stored at 4°C in sealed plastic bags until the raw dust was sieved through a 0.3 μ m screen to obtain fine dust. Fine dust (200 mg) from each of the 14 samples was then extracted in 4 ml of borate-buffered saline. After centrifugation, the supernatant obtained was passed through an 0.45 μ m filter. The resulting filtrate constituted the dust extract.

IgE antibody assays

Serum was separated from the blood samples, frozen and transported on dry ice to France where the assays were performed. Specific IgE antibodies against 12 sets of inhalant allergens were measured by a RAST-type radioimmunoassay as described by Guerin et al. [17] and Grassi et al. [18]. The extracts (listed in Table 1), representing mite, mould and plant species present in the humid zones of Europe, plus the locally prevalent Bermuda grass (Cynodon dactylon) and sugar cane (Saccharum officinarum) pollens, were supplied by Laboratoire des Stallergenes (Fresnes, France). We did not assay the sera for IgE antibody against common domestic animals since clinical histories were negative for such allergies and preliminary skin testing with cat dander extract was negative in all the subjects tested (data not shown). The D. pteronyssinus and D. farinae extracts were prepared from fresh cultures including medium containing human dander, Saccharomyces cerevisiae and hydrolysed beef liver (Allerbio, Varennes, France). The crude allergenic materials were extracted in aqueous solution. They were standardized in comparison with an NIBSC/ WHO reference preparation to contain 100 skin reactive units (IR)/ml [19].

After filtration, the extracts were coupled to paper discs activated with CNBr. The results of these assays are expressed in RAST units (RU). In fact, the RU values obtained in our laboratory are related to the International Units for total IgE since we regularly use the same monoclonal anti-IgE antibody for the determination of total serum IgE [19]. To simplify the analysis of the data, we classified the results according to the Pharmacia (P) schema: class 0 = <0.34 PRU/ml, class 1 = 0.34-0.59PRU/ml, class 2 = 0.60-3.4 PRU/ml, class 3 = 3.5-21PRU/ml and class 4 = > 21 PRU/ml.

Assays for Der P I and Der f I allergens in house dust

Measurements were performed on dust extracts by a twosite monoclonal antibody ELISA. The monoclonal antibodies (MoAbs) used in this study recognized specific and non-overlapping epitopes on *Der p* I [20] and *Der f* I [21]. For the assay of *Der p* I, 10B9F6 MoAb was coated onto microtitre plates and biotinylated 5H8C12 MoAb was used as the secondary antibody. In the *Der f* I assay, MAF 6 MoAb was the catching antibody and biotinylated MAF 9 MoAb was the detecting antibody. The procedure described by Luczynska *et al.* [22] was performed with some modifications. Briefly, 100 μ l of dust extract diluted from 1/4 to 1/500 was incubated overnight in the coated wells of the microtitre plates. After extensive washing, 100 μ l of the biotinylated MoAb (8–12·5 ng) was added to each well. Bound MoAb was detected by the

Table 1. Sets of allergens tested

Five grass mix, containing equal parts of extracts of Dactylis glomerata, Lolium perenne, Phleum pratense, Poa pratensis and Anthoxanthum odoratum.

Cynodon dactylon (Bermuda grass)

Saccharum officinarum (sugar canc)

Weed mix I, containing equal parts of extracts of Artemisia absinthium, Artemisia vulgaris, Ambrosia elatior, Xanthium strumarium, Chrysanthemum leucanthenum, Taraxacum vulgare and Solidago canadensis

Weed mix II, containing equal parts of extracts of Amaranthus retroflexus, Chenopodium album and Rumex acetosa

Weed mix III, containing 10% of an extract of Medicago sativa, 10% Trifolium pratense, 10% Brassica nigra, 10% Urtica dioica and 60% Plantago lanceolata

Mould mix I, containing extracts of Alternaria tenius, Aspergillus mix, Hormodendrum and Penicillium mix

Mould mix II, containing extracts of Botrytis cinerea, Mucor racemosus, Rhizopus nigricans and Stemphylium botryosum

Mould mix III, containing extracts of Chaetomium, Fusarium vasinfectum, Neurospora crassa and Pullularia pullulans

Mould mix IV, containing extracts of Epicoccum, Helminthosporium interseminatum, Merulius lacrymans, and Trichothecium

Dermatophagoides pteronyssinus

Dermatophagoides farinae

addition of alkaline phosphatase conjugated to streptavidin (1/5000) (Amersham, U.K.) and the enzyme activity was determined in the presence of *p*-nitrophenyl phosphate (1 mg/ml) in diethanolamine buffer. Absorbance was read 20 to 30 min later at 405 nm (Tittertek Multiscan MCC340, Flow Laboratories). Using standard dilutions, reference curves were established for each allergen in the range of 0.5 to 62.5 ng/ml (International Solution 82/518 from the National Institute of Biological Standards and Control for *Der p* I, and purified *Der f* I [23] as internal reference for *Der f* I). Results were expressed as micrograms of *Der p* I and *Der f* I per gram of fine dust. The mean interassay coefficient of variation (CV) was 15% and the intraassay CV was 5%.

Mite species identification

Samples of raw dust were examined by light microscopy as described by Fain *et al.* [9].

RESULTS

Table 2 summarizes the results of RAST assays for each of the 12 sets of allergens on the 110 sera. Seventy-five (68%) contained detectable antibody to at least one of the allergens tested. It is immediately evident that the house dust mites *D. pteronyssinus* and *D. farinae* are the most important allergens for this group of allergic Mauritians. In contrast, the great majority of the sera were negative for antibodies against the pollens and moulds.

Table 2	2. F	AST	results	on	110	sera
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Allergen	Number positive (%)		
D. pteronyssinus	66 (60.0%)		
D. farinae	58 (52.7%)		
Five grass mix	8 (7·3%)		
Bermuda grass	17 (15.6%)*		
Sugar cane	6 (5.4%)*		
Weed mix I	6 (5.5%)*		
Weed mix II	9 (8.2%)*		
Weed mix III	7 (6·4%)*		
Mould mix I	1 (0.9%)*		
Mould mix II	24 (22.0%)*		
Mould mix III	1 (0.9%)*		
Mould mix IV	1 (0.9%)*		
	<u> </u>		

* 109 sera only

For further analysis these sets of allergens were considered in three groups: (i) the pollens; (ii) the four mould mixtures; and (iii) the two mite extracts.

Considering the pollens first, we observed that there were 18 individuals ($16\cdot3\%$) with antibodies to one or more of these allergens. Half of them were sensitive to a number of different pollens and usually to allergens in groups 2 and 3 as well. As shown in Table 2, the most frequent cause of pollen allergy was Bermuda grass. On the other hand, sensitivity to the locally abundant sugar cane was rather infrequent, as was the case also for common grasses and weeds. Moreover, the amount of

Interval	<0.75	0.75->0.90	0.90->1.10	1.10->1.25	>1.25
No. of sera/range (%)	2 (1.8)	2 (1.8)	74 (67·3)	3 (2.7)	29 (26·4)

Table 3. Ratio of the anti-D. pteronyssinus to anti-D. farinae titres

anti-pollen antibody was low (Class 1) or moderate (Class 2) in all these sera except one, which had a titre of Class 4 against Bermuda grass.

Concerning the results of tests with the mould mixtures (group 2), we found that 16 sera (14.7%) had a RAST value in Class 2 and 8 (7.4%) in Class 1 against Mix II. Only one serum contained detectable antibody against any of the other three mould mixtures.

IgE antibody to mite allergens (group 3) was clearly predominant in the 110 sera that were tested: 67 were positive, including 66 (60%) to D. pteronyssinus and 58 (52.7%) to D. farinae. Comparing results obtained with the two mite allergens, we found that 41 of the 110 sera (37.3%) were strongly positive (Class 3 or 4) to both while $64(58\cdot2\%)$ were either negative or not very positive (Class 1 or 2). The five remaining sera (4.5%) were strongly positive to D. pteronyssinus but negative or only slightly positive to D. farinae. None of the sera were strongly positive to D. farinae but negative to D. pteronyssinus. As a further step in this comparison for each serum we analysed the ratio of the titre of anti-D. pteronyssinus antibody to the titre of anti-D. farinae antibody. To simplify this analysis, when a serum contained either less than 0.34 RU/ml (Class 0) to both mite allergens or more than 21 RU/ml (Class 4) to both, then the ratio was taken as 1 in both cases. The ratios were then divided into five ranges; the number of sera falling into each range is listed in Table 3. Two-thirds of the sera tested contained approximately the same amount of antibody against D. pteronyssinus and D. farinae. In the majority of the remaining sera, the titre of antibody against D. pteronyssinus was definitely greater than the anti-D. faringe titre.

Among the 81 individuals whose clinical histories were available, 60 were asthmatic. Forty-five (75%) of these asthmatic subjects had antibody to one or both of the mites. Four other asthmatics were allergic to pollen and/ or mould allergens; no antibody was detected in the sera of the remaining eleven asthmatics. In contrast, only six (35%) of the 17 subjects with a diagnosis of allergic rhinitis without asthma had detectable anti-mite antibody.

As these results showed a prevalence of specific IgE to house dust mites in allergic Mauritians, small samples of dust were collected in three houses in Port-Louis and sent to one of us (A.F.) for analysis. Table 4 lists the species as Table 4. Mite species found in house dust samples

House no. 85:

Chortoglyphus arcuatus (chortoglyphidae): 2 specimens Tropilichus aframericanus (Glycyphidae): 3 specimens Glycyphagus sp.: protonymphal skins with inert hypopi

House no. 89:

D. pteronyssinus (Pyroglyphidae): 62 specimens, mostly adults
Hirstia domicola (Pyroglyphidae): 12 specimens
Euroglyphus maynei (Pyroglyphidae): 18 specimens, mostly adults
Tropilichus aframericanus: 8 specimens
Blomia tropicalis: 4 adult specimens
Chortoglyphus arcuatus: 5 specimens
Glycyphagus sp.: protonymphal skins with inert hypopi
House no. 90:
D. pteronyssinus: 1 specimen
Tropilichus aframericanus: 6 specimens
Chortoglyphus arcuatus: 11 specimens
Glycyphagus sp.: protonymphal skins with inert hypopi.

well as the number of mites found in the dust. Mites belonging to the genera *Pyroglyphidae*, *Glycyphagidae* and *Chortoglyphiae* were identified. It should be noted especially that *D. farinae* was not found.

The concentration of the major allergens Der p I and Der f I, determined by a sandwich ELISA method using specific MoAbs, was then assessed in 14 dust samples. As shown by the curves in Figure 1a and b, 10B9F6 MoAb detects only Der p I and MAF 6 MoAb detects only Der f I. Der p I levels ranged from 1.65 to 33 μ g/g of fine dust whereas Der f I allergen was not detected in any of the samples (Figure 1c).

Discussion

Mauritius is a rather densely populated (close to 500 inhabitants/km²) small volcanic island (1865 km²) situated between latitudes 20° and 21° south in the western

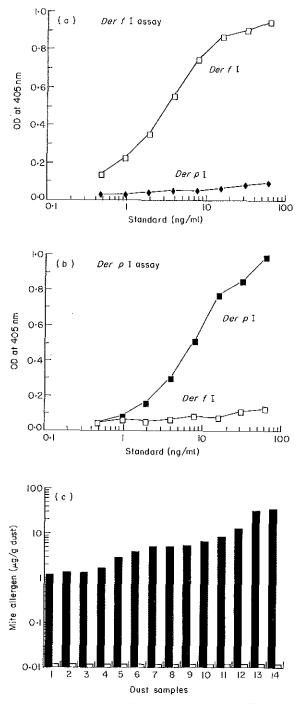


Fig. 1. Der p I and Der f I assays. (a) and (b) Typical dose response curves with standard allergen. (c) Measurements of Der p I and Der f I in house dust samples. \blacksquare , Der p I. \Box , Der f I.

Indian Ocean 700 km east of the African coast. The island is covered by small mountains, the highest reaching an altitude of 825 m. While having a generally moderate climate, the summers are very hot and humid. The island presents, in fact, a diversity of microclimates, ranging from typically tropical to nearly desert-like. Along the coastal regions, there is a narrow grassy fringe where Bermuda grass grows abundantly. On the plain the soil is very fertile, supporting the production of tropical crops, the chief among which is sugar cane. It must be noted that the type of sugar cane cultivated on Mauritius had been selected to remain sterile, i.e. to be non-pollinating.

The results of our serological and environmental survey suggest that D. pteronyssinus is certainly the most important source of allergy on this tropical island. As in other populations [1–3], nearly two-thirds of the allergic subjects we studied, and an even greater proportion of those with asthma, were sensitive to house dust mites. Since D. pteronyssinus but not D. farinae was found in the dust samples we examined, it is likely that the anti-D. farinae antibody in the 41 highly positive sera was induced in response to epitopes common to the two mite species, whereas the anti-D. pteronyssinus antibody in the five sera without anti-D. farinae antibody was probably induced in response to D. pteronyssinus-specific epitopes [24]. In addition, because none of the sera was strongly positive to D. farinae alone, it appears there was no antibody response to D. farinae-specific epitopes. The failure to find D. farinae in the dust, either microscopically or immunologically, was not surprising, as it is generally reported to be much less prevalent than other mites [25,26]. Moreover, even if it were found by a more extensive search, D. farinae does not appear to be a significant source of allergy on Mauritius.

Given the local temperature and humidity, it was also not surprising that diverse mites in addition to D. pteronyssinus were found in Mauritian house dust, E. maynei, also a pyroglyphid, has been identified in house dust from many countries [27-29] and according to Colloff et al. [29] it may be significant clinically. Another pyroglyphid mite that we observed, H. domicola, has been reported less frequently in house dust [9]. C. arcuatus, a chortoglyphid mite, is a cosmopolitan species commonly found in dust from floors of barns, stables, granaries, etc., but it has also been observed in house dust [9]. In contrast, the house is probably not the normal habitat for T. aframericanus, a glycyphagid mite, although it has been described in house dust from Brazil and Zaire [9]. The presence of mites dominant in floor dust but not normally present in bedding could be related to the largely tropicalrural environment in which the dust samples were collected. Further studies are necessary to determine the clinical importance of these various mites in the local population.

In contrast to the high prevalence of mite sensitivity, the frequency of sensitivity to other air-borne allergens was relatively low in the population we studied. Furthermore, the titres of the IgE anti-pollen and anti-mould

antibodies in their sera were, with one exception, low (Class 1 or 2), and the positive sera were from individuals who were multiply sensitive. The low frequency of antibody to pollens (18/100) might be attributed to the group of allergenic extracts that we used: the species of pollinating plants on Mauritius are not the same as those in strictly temperate zones such as Europe, thus the extracts we used did not truly represent the local flora. Another possibility is that the low titres reflect crossreactions due to antibodies induced by pollen from local plants. It is also possible that because of the frequent rain showers on the island most of the pollen from windpollinating plants is washed out of the air. Unfortunately, qualitative and quantitative data on air-borne pollen do not yet exist for Mauritius. Therefore it is possible that our data underestimate the true prevalence of sensitivity to air-borne pollens on Mauritius, with the exception perhaps of sugar cane inasmuch as a systematic investigation by skin testing and RAST on the nearby island of Reunion, where a similar race of sterile sugar cane grows, had revealed a similar low prevalence of allergy to that pollen (unpublished data). It is also clear that the allergic population sampled was small in numbers and therefore our data may not indicate the true distribution of specific sensitivities in this population.

The absence of detectable antibodies to mould allergens in Mixtures I, III and IV and the presence of antibody to Mixture II in 22% of the sera could be explained by the fact that moulds that classically induce allergy in temperate zones such as France, for example *Alternaria* (present in Mixture I), do not grow well in a humid tropical climate while *Botrytis*, *Mucor* and *Rhizopus* (present in Mixture II) develop quite well in such climates, where they are likely to contaminate debris remaining from the sugar cane harvest.

In conclusion, the presence of *D. pteronyssinus* in the local house dust and the observation that it is by far the most frequent cause of sensitization among the group of allergic subjects we studied indicate that it—and perhaps other mites as well—is the most important allergen on the island of Mauritius. Nevertheless, as the information obtained from our examination of the dust was primarily qualitative, further immunological and environmental (including air sampling) work is needed to determine the relative importance of various mites and other potential allergens for the development of allergic sensitivity in this tropical environment.

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