Reviews
Analyses

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Dust mite allergens and asthma: a worldwide problem*

INTERNATIONAL WORKSHOP REPORT[1]

After the discovery of house dust mites in 1964 their association with asthma has been reported from many different parts of the world including the developing countries. Two sets of major allergens from mites of the genus Dermatophagoides *are now well recognized. The Group I allergens are glycoproteins of relative molecular mass* (M_r) *25 000, which show both structural homology and cross-reactivity. The allergen* Der p I *has been cloned and sequenced confirming the* M_r *and establishing its nature as a protease. The Group II allergens* (M_r *15 000) show even closer homology and cross-reactivity. Specific immunoassays for Group I and Group II allergens, using monospecific antisera and monoclonal antibodies, have been standardized and are suitable for measuring allergen levels in different parts of the world.*

Measures for reducing the levels of mite allergens in houses include the covering of mattresses, hot washing of bedding, and removal of carpets from bedrooms as well as humidity control, vacuum cleaning, and the use of acaricides in the rest of the house. There is already evidence that these procedures can cause a major improvement in the symptoms of asthma. While provisional standards for both sensitization to mites and also mite allergen exposure can now be recommended, there is an urgent need for controlled studies using protocols demonstrated to reduce mite allergen levels by at least tenfold and for further international collaboration.

The role of mites of the family Pyrogliphidae as the single most important source of house dust allergens was established 20 years ago (*1*). Since then, evidence for sensitization of asthmatic patients to mite allergens has been reported from many different parts of the world (*2–7*). In addition, there has been steady

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[1] The signatories of this report are the following who participated in the International Workshop: T. A. E. Platts-Mills and A. L. de Weck (*Chairmen*); R. C. Aalberse, Amsterdam, Netherlands; J. C. Bessot, Strasbourg, France; B. Bjorksten, Linköping, Sweden; E. Bischoff, Mainz, Federal Republic of Germany; J. Bousquet, Montpellier, France; J. E. M. H. Van Bronswijk, Amsterdam, Netherlands; G. P. Channabasavanna, Andaman Islands, India; M. Chapman, Charlottesville, VA, USA; M. Colloff, Glasgow, Scotland; A. Goldstein, Washington, DC, USA; B. Guerin, Fresnes, France; B. Hart, Oxford, England; Chein-Soo Hong, Seoul, Republic of Korea; Koji Ito, Tokyo, Japan; W. Jorde, Mönchengladbach, Federal Republic of Germany; J. Korsgaard, Aarhus, Denmark; J. Le Mao, Paris, France; P. Lind, Copenhagen, Denmark; H. Lowenstein, Copenhagen, Denmark; E. B. Mitchell, Dublin, Ireland; T. Miyamoto, Tokyo, Japan; A. B. Murray, Vancouver, B.C., Canada; D. Nolte, Bad Reichenhall, Federal Republic of Germany; P. S. Norman, Baltimore, MD, USA; G. Pauli, Strasbourg, France; H. R. Ranganath, Karnataka, India; C. Reed, Rochester, MN, USA; J. Reiser, London, England; G. Stewart, Perth, Australia; K. J. Turner, Perth, Australia; D. Vervloet, Marseilles, France; and Tinghuan Wen, Shanghai, China.

progress in our understanding of the way in which allergens, particularly those derived from dust mites, can contribute to asthma, rhinitis and atopic dermatitis (*5–8*). In the last ten years many different mite allergens have been identified, the most important ones have been purified, and monoclonal antibodies have been developed against the purified allergens (*9–11*). In 1984 an International Standard (IS) for the dust mite *Dermatophagoides pteronyssinus* was adopted by the World Health Organization, which was designated as NIBSC 82/518 (*12*). Increased knowledge of the biology of mites has also allowed the development of improved protocols for reducing the quantities of mite allergens in houses. In addition, clear evidence that such measures can help patients has been obtained (*13–15*). Finally, it has recently become possible to measure mite allergens in house dust using assays which are simple enough for widespread use in research or clinical practice.

Despite many advances in pharmacological treatment, asthma remains a major clinical problem and there is evidence that both the prevalence and severity of the disease are increasing (*16, 17*). It is therefore perhaps surprising that the use of allergen-specific avoidance measures has received only limited acceptance in the management of asthma. In part this failure can be attributed to the lack of a quantitative definition of either mite allergy or mite infestation. Indeed, it remains difficult for most physicians to identify with any certainty those patients who are "at risk". The present workshop was organized because it was felt that the present state of knowledge was sufficient to establish preliminary guidelines for the levels of mite allergen in houses that should be regarded as a risk factor for disease and to establish guidelines for measuring the mite allergens in house dust. In addition, a major objective was to identify those areas where future collaborative studies would be helpful both in understanding the role of mite allergens in disease and in further defining the measures necessary to control mite infestation.

EPIDEMIOLOGY

Reports from many different countries have demonstrated a high prevalence of mite allergy among patients with asthma (Table 1). In most cases the patients were persons who had been referred to a clinic and the controls were randomly selected from the same or a similar population base. However, the reported prevalence figures among asthmatics (45–85%) and controls (5–30%) are such as to leave little doubt that sensitivity to mites is a risk factor for asthma. Formal case–control and population studies have confirmed the association. A report from Denmark suggested that the presence of more than 100

Table 1. International studies on the association of dust mite allergy and asthma[a]

Area or country	Authors and reference[b]
Europe	
Netherlands	R. Voorhorst et al. (*1*)
United Kingdom	J. M. Smith et al. (*6*) K. Maunsell et al. (*53*)
Denmark	J. Korsgaard (*18*)
France	G. Pauli et al. (*54*)
Federal Republic of Germany	E. Bischoff & W. Schirmacher (*38*)
Tristan da Cunha	J. Mantle & J. Pepys (*55*)
Tenerife	E. Fernandez-Caldas[c]
Asia	
Japan	T. Miyamoto et al. (*56*)
Hong Kong	M. Gabriel et al. (*57*)
China (Province of Taiwan)	K. H. Hsieh (*58*)
China	T. Wen et al.[d]
South America	
Colombia	M. S. Mulla & S. Medina (*73*)
Brazil	C. K. Naispitz & K. Aruda[e]
Argentina	J. Schuhl (*59*)
Venezuela	I. Hurtado & M. Parini (*60*)
North America	
Canada	A. B. Murray et al. (*61*)
USA	J. E. M. H. Van Bronswijk & R. N. Sinha (*2*) L. G. Arlian et al. (*62*) T. F. Smith et al. (*63*) T. A. E. Platts-Mills et al. (*25*)
Pacific	
Australia	A. J. Woolcock (*64*) C. W. Clarke & P. M. Aldons (*65*) C. Witt et al. (*66*)
Hawaii	J. L. Sharp & E. M. Haramoto (*67*) M. Nadchatram et al. (*68*)
Papua New Guinea	W. F. Green et al. (*69*) G. K. Dowse et al. (*21*)
Africa	
Zambia	D. J. Buchanan & I. G. Jones (*70*)
Zimbabwe	J. B. Cookson et al. (*71*)
South Africa	D. Ordman (*72*)

[a] Most, but not all, studies have reported an increased prevalence of mite allergy among asthmatic patients compared to controls. The common feature of the *negative* studies is that they have been in areas of low humidity, e.g., Davos, Switzerland (*48*), Denver, Colorado (*49*), inland California (*50*), inland New South Wales, Australia (*24*), Scandinavia (*51*), Canada (*52*), and Briançon in the French Alps (*26*). In each situation low levels of mites have been recorded in houses.

[b] In many cases the reference indicates other relevant earlier references for that area.

[c] Ph. D. thesis, 1988.

[d] Report presented at the Workshop.

[e] Personal communication, 1987.

mites/g of dust in a house should be regarded as a major risk factor for asthma and recommended that building codes should be modified to recognize this problem (*18*). Reports from many parts of the world have now suggested that a level of 100–500 mites/g dust should be regarded as a risk factor or "a maximum acceptable contamination" (*5, 18–21*). Furthermore, there is good evidence that the level of mites in houses in the USA can vary with changes in the seasons, the weather, or in the furniture of a house and that these variations can alter the risk level (*22, 23*).

At the workshop, a high prevalence of mite allergy among asthmatics associated with large numbers of mites in house dust was reported from Seoul (Republic of Korea) and from Bangalore (India). In addition, four reports were presented that focused on the risk of mite allergy in asthma:

—A high prevalence of positive skin tests to mite allergen in Shanghai (32%) was related both to high numbers of mites in pillow dust and to an increased prevalence of asthma.
—A dramatic increase in adult asthma in the South Fore region of Papua New Guinea (from 0.15% to 7.3%) was related to the introduction of blankets, which became infested with mites (mean level, 1300 mites/g dust), and the individuals who developed asthma were found to have IgE antibodies to mite allergen (*21, 24*).
—The presence of IgE antibodies to mites ($\geqslant$20 ng IgE/ml) (and also to cockroaches or cats) was a major risk factor for Emergency Room visits by patients with asthma in the USA (*25*).
—A survey in two areas of France demonstrated a strikingly lower prevalence of asthma at high altitude where the prevalence both of mites and of sensitization was lower (*26*).

These studies confirm other reports that mites representing a variety of species but predominantly those of the genus *Dermatophagoides* are found wherever local environmental conditions, such as appropriate bedding, humidity, warmth, etc., favour their proliferation. Further, these studies taken together confirm that the presence of a high level of mites can be a major risk factor for both the development of IgE antibody and the development of asthma.

Definition of sensitization

(*a*) *Skin testing.* The epidemiology of dust-mite-associated disease requires an agreed position on determining sensitization, for which both skin testing and measurements of IgE antibodies using the radioallergosorbent test (RAST) are widely used. Skin testing using the prick test technique should employ extracts that are standardized relative to established national or International Standards. Response should be assessed by measurement of wheal size, and the use of histamine as a positive control. Although variable definitions of a positive skin test have been used, there was general agreement that a wheal $\geqslant$5 mm in diameter (using a prick test and an extract containing 20–70 μg *Der p* I/ml) correlates with serum assays for IgE antibody, and can be consistently reproduced on repeat testing using a mite extract of similar potency.

(*b*) *Serum assays for IgE antibody.* Radioallergosorbent test results should be standardized by an accurate description of the extract used on the discs (i.e., source material and specific allergen content) and controlled relative to established serum pools (e.g., the international standard, NIBSC 82/528) (*12*). The ampoules of NIBSC 82/528 are considered to contain 1150 units of IgE antibody to *D. pteronyssinus* and recent estimates suggest that the unit is approximately 0.1 ng IgE antibody. We suggest that 40 RAST units (i.e., 5 ng/ml) should be regarded a a definite positive and 200 RAST units (i.e., 20 ng IgE antibody/ml) should be regarded as a high level. It is clearly necessary to relate these values to other assay systems in common use.

It was felt that the determination of IgG and IgG-subclass antibody levels, T-cell sensitization, and allergen challenge studies, while of importance to research, were unlikely to contribute to epidemiological studies. By contrast, there was general agreement that spirometry and the assessment of nonspecific bronchial reactivity (where possible) using histamine, methacholine, or cold air could be an important part of these studies, particularly as objective evidence of asthma or for monitoring the effects of allergen avoidance.

ENTOMOLOGY

The discipline of entomology has gathered substantial data on mites, which form a basis for all studies on mites and their relationship to disease.

Taxonomy

Taxonomy now lists 47 species in 17 genera in the family Pyroglyphidae; six of these (*Dermatophagoides, Euroglyphus, Hirstia, Malayoglyphus, Pyroglyphus* and *Sturnophagoides*) are common in temperate or tropical regions in the world. In Western Europe, Japan, North America, New Zealand and Australia two pyroglyphid genera (*Dermatophagoides* and *Euroglyphus*) predominate in the majority of houses (Table 1). In addition, studies were presented at the workshop documenting high incidence of *Dermatophagoides* species and *E. maynei* in houses in Seoul (Republic of Korea), Shanghai (China),

Bangalore (India), and the South Fore Province (Papua New Guinea). In any new area it is essential that house dust should be examined microscopically to establish the dominant species and these results should be considered when deciding the extracts used for skin testing or RAST. To support studies on allergen identification and mite distribution it would be helpful (*a*) to prepare a list of individuals and institutions willing to identify mites collected by fellow scientists, and (*b*) to establish a laboratory to keep and distribute pure cultures of approximately ten common species of pyroglyphid mites (instead of the three that are currently available). Further research would be required to establish such a collection of mite cultures and to prepare taxonomic keys for the identification of mites in cultures and house dust. Other mites that inhabit houses include storage mites (including *Acarus, Glycyphagus, Lepidoglyphus, Tyrophagus,* and in tropical countries *Blomia* and *Suidasia*), and also *Tarsonemids.* It will be important to define further the antigenic relationship between these mites and the pyroglyphid group; however, cultures of many of the storage mites are already available.

Physiology of allergen production

There are three different ways mites may secrete or excrete products: by egg laying, by lateral "oil" gland secretion (influencing integument permeability as well as pheromone secretion), and by faeces production (including guanine secretion). There is currently no evidence that eggs contribute allergens to house dust. Although they have not been shown to be a source of allergens, the chemistry of the secretions of the lateral glands needs further study. One of the major allergens (*Der p* I) is now known to be a protease probably related to digestion (*9, 27, 28*). Guanine is an end-product of purine digestion/excretion and can be used as a specific marker of mite infestation. Because of this it would be helpful to elucidate further details on the nutrition of mites and the chemistry of their faeces in different habitats in the home.

Physiology: relevance to controlling mite numbers

Several physiological processes of mites have been studied which could be relevant to control measures. These include juvenile hormones, (alarm) pheromones, nutritional intervention by fungi, and sterilization by X-rays. Currently these approaches have not yielded promising results; pheromones and hormones are very expensive; strategies that encourage fungal growth are likely to produce disadvantages in terms of increased fungal allergens; use of X-ray sterilized males does not promise prolonged benefit because of the short life of mites (approximately 3 months) and their constant reintroduction on clothing. Evidence that mites are dependent on fungi for reproduction led to the use of natamycin (a macrolide fungicide) as an acaricide. It is important to consider possible differences in sensitivity between mite species when using chemicals, acaricides or other control measures.

Biology (autecology)

Comparison of laboratory results with actual measurements in dwellings showed that humidity is usually the decisive limiting factor for mite growth (*18–20, 22, 23, 29, 30*). Most homes in developed countries contain at least one habitat where food and temperature are conducive to mite growth. The primary food source of mites appears to be skin scales, and/or fungi growing on skin scales, but many other food sources may be used. No viral or bacterial diseases of pyroglyphid mites have yet been studied, but the adverse effect of excessive fungal growth in mite cultures is well known. Predation by other mites, such as *Cheyletidae* or *Gamasina,* is common, but these species are not effective in controlling pyroglyphid mite populations in the home.

Ecology (synecology)

Studies of the geographical, seasonal, and intra-home distributions, as well as the ecosystem structure in different house dusts, show that pyroglyphid mites are cosmopolitan. In general, the highest numbers of dust mites (i.e., including non-pyroglyphid mites) are usually found in bedding, upholstered furniture or carpeting; and fewer species are encountered in mattresses and upholstered furniture than in carpets, in urban compared with rural areas, and in temperate as opposed to tropical countries.

Over the last 50 years there have been several changes in construction and furnishing of houses in developed countries which would foster mite proliferation. These include (*a*) introduction of vacuum cleaners, so that carpets are no longer picked up and beaten but are often permanently laid down from wall to wall; (*b*) installation of central heating, so that rooms other than the bedrooms are maintained at a temperature conducive to mite growth, which is optimally 17–25 °C; (*c*) use of cool water detergents to wash bedding (this does not harm live mites); (*d*) reduced ventilation for energy conservation increases the indoor humidity; and (*e*) use of air humidification systems that maintain relative humidity at $>50\%$. By contrast, air conditioning usually reduces the humidity and may be efficient at controlling mite growth.

MITE ALLERGENS

Identification and standardization

In recent years, two major groups of allergens from the genus Dermatophagoides have been defined. The Group I allergens (*Der p* I, *Der f* I, *Der m* I) are heat-labile glycoproteins (relative molecular mass, M_r, 25 000), which are heterogeneous on isoelectric-focusing (IEF) (pI, 4.7–7.4) and are excreted in faeces (*5*, *9*, *10*, *27*, *31–33*). These allergens appear to be structural homologues and have very similar *N*-terminal amino acid sequences. The Group II allergens (*Der p* II and *Der f* II) are proteins (M_r, 15 000) and also heterogeneous on IEF, with almost identical *N*-terminal amino acid sequences (*5*, *11*, *34*).[a] The gene encoding *Der p* I has been cloned and expressed in lambda gt 11 and the full amino acid sequence has been deduced from the cDNA (*28*). The majority of mite allergic individuals produce IgE antibody to both the Group I and Group II allergens. While there is extensive cross-reactivity between *Der p* I, *Der f* I and *Der m* I (and also between *Der p* II and *Der f* II), there is no evidence for structural similarity or cross-reactivity between the Group I and Group II allergens. Other mite allergens have also been identified, but their allergenic importance remains to be established (*35*). Several laboratories have produced murine monoclonal antibodies (Mabs) to the Group I and Group II allergens (*5*, *36*, *37*). These Mabs have been used for allergen purification, epitope mapping studies, and to develop specific immunoassays for allergen quantification (*37*). In addition, monospecific polyclonal rabbit antibodies have been produced which recognize either species-specific or cross-reacting epitopes on the Group I allergens (*5*).

It is essential that assays of allergens are related to an established national or international standard extract. The best established and characterized extract is the WHO International Standard (NIBSC 82/518), which is 4000 ampoules of freeze-dried glass-sealed extract made from whole-mite culture (*12*). This standard was assigned a potency of 100 000 International Units. The ampoules contain 12.5 μg *Der p* I and 0.5 μg *Der p* II. Other standards include those produced by the Food and Drug Administration (FDA) in the USA, which are made from isolated mite bodies. The FDA *D. pteronyssinus* standard contains 46 μg *Der p* I/ml and 25 μg *Der p* II/ml. The FDA *D. farinae* standard contains 35 μg *Der f* I/ml and 16 μg *Der f* II/ml. We are grateful to Dr Peter Heymann (University of Virginia) for the provisional estimates of Group II allergen content which were made using a two-site Mab assay. The striking difference in ratios of Group I to Group II allergens is a feature of mite extracts made from different source materials. In the future it may be advisable to have separate standards or substandards made from different source materials. However, the most important role of these standards is to act as a stable reference point over a long period (i.e., more than 10 years) and the WHO International Standard is the best established for that purpose.

[a] AALBERSE, R. A. Development of a monoclonal antibody to the dust mite allergen *Der p* II (DpX). (Unpublished paper).

Current and future studies

Further sequencing analyses of the Group I and II allergens will help to establish the degree of structural homology within the groups. This will be important for research studies on the immune response to mite allergens in different groups of patients. Although the data suggest that the Group I and II proteins are the two dominant mite allergens, further work is needed to establish the significance of other *Dermatophagoides* allergens. It is also of crucial importance to identify and study allergens from *Euroglyphus maynei*, since this species has a worldwide distribution, can be dominant in houses, and represents an important subgroup of the pyroglyphidae.

MITE ASSESSMENT METHODOLOGY

There are three types of methods for estimating mite concentration in homes: mite counts, immunochemical assays of mite allergen, and guanine determinations. The choice of a particular method will be dictated by the specific purpose of the study.

Mite counts

The prevalence of mites can be determined by counting them under a microscope after separation from a dust sample by flotation or other methods. This technique permits species identification of the predominant mites and recognition of live, dead, larval or adult types. Disadvantages include (*a*) the need for training and skill in recognizing different mite species, (*b*) inability to assess faecal particles, (*c*) difficulty in extracting mites from fabrics, and (*d*) unsuitability (i.e., too slow) for large-scale studies.

Immunochemical assays

Mite allergens can be quantified in extracts of house dust by several techniques. Inhibition radioimmunoassays using human IgE have the advantage of measuring "relevant" antigenic determinants that have elicited a response in allergic subjects. Disadvantages of the RAST inhibition technique include variability in antibody specificity in different sera,

and relatively low sensitivity.

Sandwich radio- or enzyme immunoassays employ either rabbit polyclonal or mouse monoclonal antibody for capture, and affinity purified antibody or a second monoclonal antibody for detection. These assays are more sensitive than RAST inhibition and particularly those using monoclonal antibodies have great potential advantages in long-term reproducibility. Other advantages of immunochemical assays are their specificity (indeed, monoclonal antibody assays can be used to distinguish the species of mites), the results can be expressed in absolute units of a defined protein, and convenience for large-scale surveys because the assays can be automated.

The disadvantages of immunochemical assays are that they require trained laboratory technicians and sophisticated laboratory equipment; even using enzyme-linked reagents the procedures may be difficult to apply in less technologically developed countries.

Guanine determination

Guanine is an excretion product of arachnids and these organisms are the most important source of guanine in house dust. Among arachnids, mites are overwhelmingly more abundant than spiders. Thus, determination of guanine in house dust is an indirect method of counting mite faecal pellets. Its advantages are simplicity and economy. The test can be performed by untrained personnel and is already commercially available in some countries (*38*, *39*).[b] Disadvantages include the inability to distinguish between mite species, the semiquantitative nature of the kit, and occasional false-negative and false-positive results.

Procedure for collecting and processing dust samples

Vacuum cleaners used for collecting dust samples can be equipped with a special attachment to collect dust onto a filter (e.g., linen or tissue paper), or the dust can be collected directly into a paper bag. Sampling time should be standardized and 2 min/m^2 has been commonly used.

Sampling sites should be consistent and preferably the following sites should be sampled separately.

(1) The upper mattress surface (roughly 2 m^2) should be vacuum cleaned for 2 min unless a shorter time provides a sample of $\geqslant$200 mg. The sampling should be spaced out rather than concentrated in a single area. If the mattress is covered with plastic the bedding should be sampled but results are not directly comparable with mattresses.

(2) Floor samples should be collected from an area of 1 m^2 in the bedroom immediately underneath and beside the bed.

(3) In the living rooom, i.e., the most occupied room away from the bedroom (except for the kitchen), the carpet should be sampled in a sufficiently large exposed area (e.g., 1 m^2 for 2 min). Living-room samples can also be obtained from upholstered furniture, but the results may be different from those in carpets. Alternative techniques of obtaining dust samples include shaking blankets in a plastic bag, using a hand-held brush to sweep surfaces and scraping flat surfaces higher than floor level with a piece of firm card, but these are less effective than vacuuming.

Large particles should be removed from dust before processing. This can be achieved by sieving through a 300 μm-mesh sieve, although most mattresses and bedding samples do not require sieving. The objective is to obtain a sample of fine dust that can be accurately weighed, though dust samples may still vary in density after sieving.

For immunochemical analysis the normal conditions for extracting the dust are in the ratio of 100 mg to 2 ml of buffered saline (or 50% glycerine), extraction being facilitated by rotation or agitation for 4 hours. The extract is then stored frozen or in 50% glycerine at -20 °C. Lyophilization or repeated freezing and thawing should be avoided. It is important to realize that allergen production from a carpet is a dynamic process, so that the rate of production per m^2 would be an ideal measurement. However, the sampling techniques involve considerable errors in the total quantity of dust collected. Therefore in almost all studies it is preferable to express the results simply as μg (or International Units) of allergen per g of dust. Similarly, mite counts are best expressed per gram of dust.

Airborne samples. Several techniques have been described for volumetric sampling using membrane filters to capture airborne particles and immunochemical assays for allergens. Mites themselves are not seen in these samples. These techniques have the advantage that they measure allergens in the air and so may be more representative of exposure than assays of settled dust. The concentration of mite allergen in the air is a reflection not only of the concentration in settled or surface dust but also of ventilation and/or domestic disturbance (*40*, *41*). A practical disadvantage of the technique is that long sampling periods (2–24 hours) are required so that short peaks of high exposure associated with disturbance in the room are not identified. At present, airborne sampling has not been shown to be superior to floor or mattress dust samples as a primary measurement of mite infestation.

[b] VAN BRONSWIJK, J. E. M. H. et al. Evaluing mite allergenicity of house dust by guanine quantification. (Unpublished paper).

MITE CONTROL

The efficacy of avoidance measures is supported by studies on patients living in mite-free environments such as high altitude or mite-free rooms where patients have shown both improvement of asthma and reduced non-specific bronchial reactivity (*5*, *42*, *43*). Several controlled studies have been unable to demonstrate an effect of simple hygienic measures either on clinical symptoms or on the density of mites (*44*, *45*). However, two controlled studies using possibly more extensive measures have demonstrated the clinical efficacy of cleaning measures in patients' houses (*14*, *15*).

Indications

Candidates for prevention measures should be identified on the basis of objective diagnosis of mite allergy (skin testing or RAST). For patients with multiple sensitivities, e.g., to cat, pollen or fungi in addition to mite, it is essential to consider specific measures for other allergens as well. The measures proposed should be related to both the severity of the patients' disease and the economic circumstances of the family. Where possible, quantitation of the severity of mite infestation should be made before recommending intervention.

Bedding, furniture and carpets

Although the bedroom is generally considered the most important room in the house, other parts of the house must be considered, both because mite allergen levels in the bedroom may be related to the other parts of the house and because the highest levels may be found in carpets, furniture or clothing elsewhere in the house (*19*, *22*, *41*).

Regular vacuum cleaning (i.e., weekly) of carpets and upholstered furniture is essential to prevent accumulation of surface dust but intensive cleaning (i.e., daily) does not give greater benefit. Other measures such as covering the mattresses with a zipped cover, or changing old heavily infested mattresses and pillows are favoured to reduce mite numbers in beds and upholstery. Bedding, including pillows and mattress pads, should be washed regularly, and it has been shown that washing should be at $\geqslant$55 °C ($\geqslant$130 °F) in order to kill the mites. In bedrooms the carpets should be removed, otherwise it is extremely difficult to control infestation of the bedding without using acaricides. With these measures, mite infestation in bedrooms can be controlled and the major remaining problem is with carpets and upholstered furniture elsewhere in the house.

Some authors regard reduction in humidity as the primary method of controlling mite allergens and go further to imply that it is difficult to control mites without reducing humidity (*18*, *44*). In any area the sources of humidity must be analysed as the relevant control measures are strikingly different in different climates and cultures. In many inland areas, as well as in the northern areas of Europe (e.g., Sweden) and the northern states of the USA (e.g., Minnesota), owing to climatic conditions, mite growth is limited in most well-ventilated houses (see Table 1 footnote). However, modern energy-saving buildings constructed with reduced ventilation may be associated with increased indoor humidity and consequent mite growth. Experience from Denmark shows that excess humidity is in some cases related to a faulty construction (ground humidity), and in other cases to low ventilation. In the latter cases, excess humidity is easily controlled by increased ventilation. In many hotter climates, outdoor absolute humidity can rise as high as 15 g/kg and, under these conditions, the indoor humidity can only be decreased by dehumidifiers or air-conditioning. Clearly these measures are not always feasible in developing countries. However, in many tropical countries the design of houses is very different, with very little carpeting, upholstered furniture or bedding so that there may not be suitable mite nests. Finally, in some areas the major source of water in houses/apartments is related to the number and activities of the occupants, e.g., water pans and cooking. At present, available data suggest that 7 g/kg is the level of absolute humidity above which excess mite growth will occur; in some areas, maintaining indoor humidity at below this level is possible and should be considered.

Acaricides

Several acaricides have been proposed. For example, solidified benzoic acid esters (the chemical used to kill scabies mites), pirimiphos methyl (widely used for killing storage mites and mosquitos), and liquid nitrogen have been shown to reduce mite numbers in mattresses and/or other textile materials during controlled studies (*5*, *46*). Many chemicals including natamycin, synthetic pyrethrins, and a mixture containing benzoic acid, thymol, terpineol and alcohols have been shown to kill mites in the laboratory. In general, these acaricides have no direct effect on the allergens already present in the carpet; by contrast, tannic acid is reported to denature the allergens while having no effect on mites. With all acaricides it remains essential to establish both their safety for widespread use and the way they should be combined with other measures as part of an overall protocol.

RECOMMENDATIONS AND GUIDELINES

Development of methodology

(1) *Identification of mites species.* The paucity of mite species which are cultured and available makes it difficult to study the relationship between species and in particular to define the specificity of allergen assays or monoclonal antibodies. Although there may be as many as twenty species that are of major importance in house dust in some parts of the world only three or four are in culture and available. It is essential to culture more of the important species of dust mites and to obtain better information about the availability of cultures. It is possible to suggest a list in order of priority, e.g., *Euroglyphus maynei, Hirstia domicola, Tarsonemus granarius, Blomia tropicalis.* It may only be necessary to maintain cultures of these species long enough to establish freeze-dried standards as well as secondary reference reagents such as monoclonal antibodies and perhaps a DNA library.

The current acarological textbooks and published articles commonly used for identifying mites are not ideal for pyroglyphid mites (*47*). This results in a tendency to identify closely related species as either *E. maynei* or as *Dermatophagoides* species. There is an urgent need for a revised guide specifically for use in identifying mites in house dust.

(2) *Monoclonal antibodies and purified allergens.* The investigation of mite allergens has been greatly facilitated by exchange of reagents between different investigators. We strongly suggest that purified allergens, monoclonal antibodies and cDNA clones are made available for research purposes via distribution through national and international agencies, e.g., the National Institutes of Health in the USA, ATCC (American Type Culture Collection), and the National Institute for Biological Standards and Control in England. In addition, we believe that monoclonal antibodies to dust mite (and other) allergens should be registered with established monoclonal antibody data bases.[c, d] There is also a need for a range of Mab directed against cross-reactive epitopes of both Group I and Group II allergens, which would be suitable for assaying allergens derived from a wider range of pyroglyphid mites.

(3) *Specific allergen assays.* Measurement of both Group I and Group II allergens may be necessary for accurate standardization of dust mite allergen extracts (*12*). However, since both these allergens are constitutively produced by mites, measurement of either Group I or Group II allergens can be used to assess the indoor levels of mite allergens. At present, it is easier to measure Group I allergens because the reagents are more widely available and the results are easier to compare with previous studies. However, there may be advantages in measuring Group II allergens, because they are less diverse structurally, more cross-reactive, and appear to be slightly more stable than Group I proteins. In all cases the results should be referred to the IS for *D. pteronyssinus* and to other mite standards as they become available.

Studies suitable for international collaboration

Avoidance studies. Using methods that have already been developed (or are being developed) we believe it is possible to achieve tenfold or greater reductions in mite allergen concentration in many houses. In bedrooms this would require the covering of mattresses, hot washing of all bedding at least every 10 days, and removal of carpets and upholstered furniture. In other parts of the house, removing carpets, changing furniture, reducing humidity, and using liquid nitrogen treatment and/or acaricides may all play an important role. Protocols for mite eradication that have a demonstrated ability to reduce mite allergen levels (preferably by at least tenfold) must be studied in controlled trials to establish their clinical effectiveness. It is essential that allergen reduction should be monitored with quantitative assays that will yield specific information about both the absolute and the relative (i.e., percentage) reduction in mite allergens necessary to reduce symptoms, medication usage and/or bronchial hyper-reactivity.

In some specific situations dramatic reductions in allergen exposure have been achieved by rehousing families from damp housing to dry apartments in Denmark, and by removing or replacing mite-infested blankets in Papua New Guinea.

Quantitative definition of the risk factors for acute or severe asthma (including mite allergen exposure). In studying the association between a disease that fluctuates in severity from time to time (i.e., asthma) and a provoking factor that is non-seasonal or semi-seasonal (e.g., mite allergens) it is important to define the point of time of the study. Studying patients at the time of increasing or severe symptoms has the advantage that the disease can be defined in terms of lung function and acute response to bronchodilators; several types of study may be fruitful:

(*a*) Patients referred to, or presenting in a clinic at a time of increasing asthmatic symptoms can be studied and the data compared with those obtained from randomly selected controls.

(*b*) Mite allergic patients with asthma can be moni-

[c] TOVEY, E. R. & BALDO, B. A. An on-line computer data bank of monoclonal antibodies that recognize allergens—invitation for entries. (Unpublished paper).

[d] Hybridoma Data Bank. A data bank on cloned cell lines and their immunoreactive products, 12301 Parklawn Drive, Rockville, MD 20852–1776, USA.

tored for a period of time and studied when their symptoms increase.

(*c*) Patients entering a hospital or other emergency room for treatment can be matched with patients presenting with an unrelated disease.

There are many other situations that may prove useful; however, for the results to be interpreted by other groups it is necessary to define the severity of asthma and the time of study, the evidence for sensitization to mite or other allergens, and the levels of allergen in their environment within a short time, i.e., 3 weeks of the clinical information. The question is whether it is possible to confirm that the *combination* of sensitization and exposure to a given level of mite allergen is a major risk factor for acute or increasing asthma.

Studies on the relationship between mites, mite allergy, and the prevalence of asthma in a community. In some population studies the prevalence of sensitivity to mites among young asthmatic patients has been very high, i.e., $\geqslant 70\%$. Under these circumstances a simple calculation of the population-attributable risk suggests that a major proportion of asthma may be directly attributable to mite allergy. In turn this implies that in an area where high levels of mites are present in houses, not only will many asthmatics have IgE antibodies to mite allergen but the prevalence of the disease may be higher (*24*, *26*). It already appears likely that mite allergy and associated asthma are more prevalent in areas where the average mite allergen levels are higher. In some studies the individual houses of allergic patients have been found to have higher levels of mites (*18*), but this has not been found in other studies.

These studies require a population survey with evaluation of asthma by history and lung function, evidence of sensitization (i.e., skin tests of IgE antibody), and finally a random survey to examine dust from houses including asthmatics and controls. The objective is not only to answer whether mite allergens influence the prevalence of asthma, but to define the levels of mites and mite allergens in houses which make them a risk factor.

Provisional guidelines for mite allergen exposure

From the preceding discussion it is possible to identify several ways in which mites can contribute to disease and the critical level for each is not necessarily the same:

(1) the level of mite allergen exposure that is a risk for inducing sensitization (as judged by skin tests of IgE antibody);

(2) the level of mite allergen in houses that is a risk for increasing the prevalence of symptomatic asthma in a community;

(3) the level of mite allergen in houses that is a risk for acute or severe attacks of asthma among mite allergic individuals;

(4) the quantitative change in mite allergen necessary to produce a significant clinical improvement.

Clearly any proposed standard or guideline will only be applicable to a majority of patients since individual patients vary enormously in their sensitivity and the most sensitive patients may well react to very low levels of allergen. However, there seems to be sufficient data to propose that 2 μg *Der p* I/g of dust (equivalent to 100 mites/g or 0.6 mg guanine/g) should be regarded as representing a risk for the development of IgE antibody and asthma. A higher level of 10 μg *Der p* I/g (equivalent to 500 mites/g dust) should be regarded as a risk factor for acute attacks of asthma and a level at which most mite allergic patients will experience symptoms. On the basis of less complete data it appears reasonable to suggest that effective reduction in symptoms will require a reduction of allergen exposure of tenfold, and that levels should at least be reduced below 2 μg *Der p* I/g of dust (or 100 mites/g of dust).

CONCLUSIONS

1. Since the discovery of house dust mites in 1964 the association between dust mite allergy and asthma has been reported from many different parts of the world including the developing countries. Both climatic (temperature and humidity) and local factors (changes in living conditions) can influence not only mite growth but also the prevalence of sensitization to mites and the prevalence of the associated asthma.

2. There is clear evidence from avoidance studies, population surveys and allergen challenge that exposure of allergic patients to mite allergens is a major cause of non-specific bronchial hyper-reactivity.

3. Two sets of major allergens from mites of the genus *Dermatophagoides* are now well recognized. The Group I allergens are glycoproteins of M_r 25 000, which show both structural homology and cross-reactivity. The allergen *Der p* I has been cloned and sequenced confirming its relative molecular mass and establishing its nature as a protease. The Group II allergens of M_r 15 000 show even closer homology and cross-reactivity.

4. Specific immunoassays for Group I and Group II allergens have been developed using monospecific antisera and monoclonal antibodies. These assays are standardized relative to the *D. pteronyssinus* International Standard and are suitable for measuring allergen levels in different parts of the world. There is also agreement about minimum methods for

sampling and extracting dust to establish the levels of infestations in a house.

5. With our present understanding of mite biology it is possible to define protocols for reducing the levels of mite allergens in houses. These include covering of mattresses, hot washing of bedding, and removal of carpets from bedrooms, together with controlling the humidity, vacuum cleaning, changing the furniture, and using acaricides in the rest of the house. There is already evidence that procedures which reduce mite allergen levels can cause a major improvement in symptoms of asthma. However, there is an urgent need for further controlled studies using protocols that have been shown to reduce mite allergen levels by tenfold or more.

6. With our present understanding and technical progress it is both possible and highly desirable to carry out international collaborative studies on the relationship between mite allergen levels and asthma. These studies should include:

—population surveys on the relationship between mite sensitivity, mite allergen exposure, symptomatic asthma and bronchial reactivity;
—case–control studies on unselected patients presenting with acute asthma;
—longitudinal studies on the relationship between exposure and symptoms in mite allergic individuals.

7. Finally, it is now possible to recommend provisional standards for both sensitization to mites and also mite allergen exposure. It is proposed that a level of 2 μg *Der p* I/g of dust (equivalent to 100 mites/g or 0.6 mg guanine/g of dust) should be regarded as a risk factor for sensitization and the development of asthma. The higher level of 10 μg *Der p* I/g of dust (or 500 mites/g of dust) is proposed as a major risk factor for the development of acute asthma in mite allergic individuals.

RÉSUMÉ

LES ALLERGÈNES D'ACARIENS DE LA POUSSIÈRE DE MAISON ET L'ASTHME: UN PROBLÈME MONDIAL

Depuis la découverte des acariens de la poussière de maison en 1964, l'association entre l'allergie aux acariens de la poussière et l'asthme a été confirmée dans différentes parties du monde, y compris les pays en développement. Les facteurs climatiques (température et humidité) et locaux (modifications des conditions de vie) influencent non seulement la prolifération des acariens mais également la prévalence de la sensibilisation à ces acariens et de l'asthme qui y est associé.

Deux groupes d'allergènes majeurs des acariens du genre *Dermatophagoides* ont été caractérisés. Les allergènes du groupe I sont des glycoprotéines de masse moléculaire relative (M_r) 25 000, qui présentent une homologie de structure et une réactivité croisée. L'allergène *Der p* I a été cloné et séquencé et la séquence observée confirme sa masse moléculaire relative ainsi que sa nature de ferment protéolytique. Les allergènes du groupe II ont une M_r de 15 000 et montrent également entre eux une homologie et une réactivité croisée. Des titrages immunologiques spécifiques ont été mis au point pour les allergènes du groupe I et du groupe II et font appel à des antisérums monospécifiques et à des anticorps monoclonaux. Ces titrages sont standardisés et permettent de mesurer les quantités d'allergènes domestiques dans différentes parties du monde. On note également un consensus quant aux méthodes utilisées pour récolter la poussière de maison et en préparer des extraits, afin de déterminer le niveau d'infestation par des acariens dans une habitation.

Les connaissances actuelles sur la biologie des acariens permettent de définir des protocoles visant à réduire le niveau d'allergènes dans les habitations. Il s'agit par exemple de mesures telles que recouvrir les matelas, laver la literie à l'eau chaude, supprimer les tapis dans les chambres à coucher, lutter contre l'humidité, éliminer la poussière à l'aspirateur, modifier le mobilier et utiliser des acaricides dans le reste de l'habitation. Il apparaît déjà que ces mesures, en réduisant le niveau d'allergènes d'acariens, peuvent améliorer de façon considérable les symptômes de l'asthme. Il est également possible désormais de recommander des normes provisoires aussi bien pour la sensibilisation aux acariens que pour l'exposition aux allergènes d'acariens. Toutefois, il est urgent d'entreprendre de nouvelles études contrôlées, avec des protocoles capables de réduire d'au moins 10 fois les niveaux domestiques d'allergènes d'acariens.

Compte tenu du niveau actuel des connaissances et du progrès technique, il est à la fois possible et extrêmement souhaitable de procéder à des études supplémentaires, basées sur une coopération internationale, sur la relation entre les niveaux domestiques d'allergènes d'acariens et l'asthme.

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