

Editor's note: The proceedings of an international workshop on dust mites were previously published in THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (1989;83:416-27). Described below are summary data from a second workshop held in 1990. I feel that this information will be of considerable interest to many of our readers because of the greatly increased investigation into the role of mite sensitivity in allergic diseases.

Dust mite allergens and asthma: Report of a second international workshop

A second international workshop on dust mite allergens and asthma was held in Minster Lovell, England, in September 1990 with the objective of reviewing both the recommendations made in 1987 and progress made since that time.¹ Epidemiologic studies from Germany, France, Australia, England, and the United States have confirmed that levels of mite exposure of 2 µg or 10 µg of group I allergen per gram (equivalent to ~100 or 500 mites per gram) of dust are relevant to asthma.²⁻⁷ The success of these studies not only supports the previously proposed threshold values but confirms that the quantitative techniques for evaluating mites or mite allergens provide a valid "index of exposure," which can be used for risk evaluation. These and previous results support the use of immunoassays on dust samples from reservoirs in the house (i.e., mattress and carpets) as the primary method of quantitating exposure.

During the last 10 years, asthma has become generally recognized as characterized by inflammation of the bronchi. This recognition has stimulated increased interest both in the use of anti-inflammatory drugs and in identifying and controlling the causes of inflammation. The necessity of identifying the causes of

Abbreviations used

MAB:	Monoclonal antibody
AD:	Atopic dermatitis
PCR:	Polymerase chain reaction
HLA:	Human leukocyte antigen

asthma is underscored by evidence from the United States, Australia, New Zealand, and the United Kingdom that the morbidity and mortality associated with asthma is increasing.⁸⁻¹¹ The therapeutic significance of identifying specific causes of asthma relates to both avoidance of the relevant allergens and immunotherapy. As far as mites are concerned, both these areas have been the subject of intensive research. There are continuing studies on traditional forms of immunotherapy, to determine not only how effective immunotherapy is in asthma but also whether high levels of exposure to indoor allergen changes the response to treatment or increases the risk of reactions. At the same time, there has been progress toward identifying B cell and T cell epitopes of mite allergens. A major objective of those studies has been to identify modified molecules or fragments of the molecules that could be used in immunotherapy. Studies have continued on techniques to reduce mite-allergen exposure. These studies range from humidity control, methods of covering mattresses, and cleaning techniques, to a range of specific acaricides.

The Minster Lovell Workshop evaluated recent developments in immunochemistry, molecular biology, and T cell biology relevant to mites, as well as the progress in studies of mite-allergen exposure and asthma. In addition, the evidence for a role of dust mite allergens in AD was considered. There are important parallels between the studies on exposure and avoidance that are relevant to AD. Studies on the response of the skin to prolonged mite-allergen exposure also provide an important model of the ways in which allergens give rise to chronic inflammation. Our objective in the present article is to outline the progress made, discuss the implications of the results,

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TABLE I. Physiochemical properties of group I, II, and III mite allergens

Allergen	MW*	Function	pI	E 1%† (1 cm)	Sequence‡	Epitopes defined by MAbs
Group I						
<i>Der p</i> I	25,000	Cysteine protease	4.5-7.1	10.0 (17.0)	cDNA	5
<i>Der f</i> I	25,000		4.7-7.2	15.6	cDNA	4
<i>Der m</i> I	25,000		—	—	N-terminal	1
<i>Eur m</i> I	25,000		—	—	PCR	—
Group II§						
<i>Der p</i> II	14,000	Unknown	7.6-8.5	6.6	cDNA	4
<i>Der f</i> II	14,000		7.8-8.3	5.8	cDNA	4
Group III						
<i>Der p</i> III	30,000	Trypsin	4->8	—	N-terminal	—
<i>Der f</i> III	29,000	Serine protease	4.1-4.7	—	N-terminal	3

MW, Molecular weight; pI, isoelectric point.

*Based on gel filtration, sodium dodecyl sulfate–polyacrylamide gel electrophoresis, or amino acid sequence analysis. The cDNA clones for *Der p* I and *Der p* II encode proteins of molecular weight 25,371 and 14,129, respectively.

†Extinction coefficient for *Der p* I (10.0) obtained by Dr. Wayne Thomas. All other values are from Yasueda et al.²⁶

‡N-terminal amino acid sequences (20 to 40 residues) have been determined for all the allergens listed. The cDNA sequences of *Der p* I and *Der p* II suggest that both allergens contain three disulfide bonds.

§*D. microceras* extracts also bind in MAb immunoassays for group II allergens, suggesting the existence of a group II homologue in this species, which has not as yet been purified.²⁴

||From Stewart et al.⁴⁰

and provide some guide to those areas that appear most promising for future studies.

TAXONOMY

The term house dust mites applies to mites of the family Pyroglyphidae, of which 10 species have been reported to occur in house dust more often than just occasionally. Four species dominate all others and are currently kept in culture: *Dermatophagoides pteronyssinus*, *D. farinae*, *D. microceras*, and *Euroglyphus maynei*. Other mites can occur in houses, and these include several species usually regarded as storage mites (e.g., *Lepidoglyphus destructor*, *Acarus siro*, and *Tyrophagus putrescentiae*), as well as *Blomia tropicalis* and species of the families Tarsonemidae and Cheyletidae.¹²⁻¹⁵ The evidence that these mites are significant comes from studies with specific extracts for skin tests and/or RAST assays. It is proposed that the term "domestic mites" should be used to include both the pyroglyphid mites and other mite species that are found in house dust and cause the development of IgE antibody responses.¹⁶ There are acarologists in many countries who are able and willing to identify and count mites found in house dust (including Drs. F. Th. M. Spieksma, M. J. Colloff, T. Wen, B. J. Hart, A. Fain, D. Baggio, L. G. Arlian, E. Fernandez-Caldas, F. Ottoboni, KY Mumcuoglu, and J. E. Van Bronswijk). Microscopic analysis of mites found in house dust is essential for precise speciation of "domestic mites." Several groups have assessed cross-

reactivity between storage mites and pyroglyphid mites with different results.¹⁷⁻²⁰ The conclusion of the conference was that there are antigens that demonstrate cross-reactivity between these disparate groups of mites; however, under circumstances in which patients are primarily exposed to nonpyroglyphid mites, the bulk of the IgE is not cross-reactive with antigens derived from *Dermatophagoides*.

An identification key has been prepared to allow nonacarologists to identify mites found in house dust.²¹ A database containing worldwide distribution and population densities of 10 common mite species is currently being prepared by Dr. Colloff and will include local environmental data and allergen concentrations. Identification of mite species is important to verify the results of allergen and guanine assays and is especially important for (1) verification of species in cultures before allergen extraction and (2) surveys of previously unstudied geographic locations.

IMMUNOCHEMISTRY, MOLECULAR BIOLOGY, AND IMMUNE RESPONSE

Allergen identification and definition

Considerable progress has been made with immunochemical techniques to identify and define mite allergens. These strategies, involving purification of individual proteins and analysis of their allergenic activity by skin testing or serologic techniques, were successful in defining the group I, group II, and group III allergens (Table I).²²⁻²⁶ The production of MAbs

to mite allergens has significantly improved allergen purification and quantification. Several groups have produced MABs to *Dermatophagoides* spp allergens, and the production of MABs to *B. tropicalis* and *L. destructor* was reported at the meeting.^{27, 28} The application of recombinant DNA technology has also provided new approaches to allergen identification by use of IgE antibodies to screen mite cDNA libraries or use of the PCR for allergen sequencing. It is important that allergens continue to be defined both in terms of protein purity, primary sequence, and reactivity with MABs, and by assessing the prevalence of specific IgE (and IgG) antibodies in mite-allergic patients. Antibody prevalence can be assessed by immunoassays or immunoblotting; however, biologic activity should be confirmed, when this is possible, by skin testing. It is also possible to assess immune responsiveness to mite allergens by comparing T cell responses and lymphokine production.

There is extensive structural and immunochemical data on the group I and group II allergens, and >80% of mite-allergic patients have IgE antibodies to these proteins. The relative importance of the group III allergens needs to be clarified, particularly since differences in the prevalence of IgE antibodies to *Der p* III and *Der f* III have been reported. Thus, 60% to 70% of mite-allergic patients have IgE antibody to *Der p* III detectable by immunoblotting and RAST, but quantitative RIA demonstrated that only 16% of patients had IgE antibody to *Der f* III.^{24, 29} There is still a need to identify other allergens and to determine their importance in terms of sensitization, exposure, and allergic symptoms. Indeed, ~30 components in *D. pteronyssinus* and *D. farinae* extracts have been identified that bind IgE antibodies.²⁹ Three of these components have recently been defined further: mite amylase, a 60 kd protein purified from *D. pteronyssinus* by Lake et al.³⁰ that reacted with IgE antibodies in 25% to 46% of sera from mite-allergic patients on immunoblotting, a 27 kd serine protease, reacting with 36% of sera, and a 14 kd protein, cloned from a *D. pteronyssinus* cDNA library by Tovey et al.,³¹ which reacted with IgE antibodies in 40% to 50% of sera. Recent studies by O'Hehir et al.³² on T cell-reactive antigens also identified mite-allergen components of 58, 39, 22, and 10 kd. At the conference, mite amylase was considered to be sufficiently well characterized as to recommend that this protein and its analogs should be regarded as group IV mite allergens.

Molecular biology

Structural and molecular studies are essential to improve our understanding of the immune response

to mite allergens. An added impetus to these studies has come from the possibility of developing more specific immunotherapeutic approaches with recombinant allergens or synthetic peptides. During the past 3 years, there have been significant advances which may be summarized as follows:

1. cDNA libraries have been prepared from *D. pteronyssinus* and *D. farinae* mRNA, and the cDNAs encoding *Der p* I, *Der p* II, *Der f* I, and *Der f* II have been cloned and sequenced.³³⁻³⁷ At the meeting, Dr. Hart presented the sequence of *Eur m* I, which had been obtained by PCR from *E. maynei* DNA.³⁸
2. The deduced amino acid sequences demonstrate 81% homology between the group I *Dermatophagoides* sp allergens and 88% homology between the group II allergens. The group I allergens demonstrate sequence homology to cysteine proteases and have functional enzymatic activity.^{33, 39, 40, 40a, 40b} *Der p* II and *Der f* II do not demonstrate homology to other proteins in the data banks, and their function is not known. N-terminal amino acid sequencing demonstrate 75% homology between *Der f* III and *Der p* III and also demonstrate that these proteins are likely to be trypsin.^{24, 40, 40a, 40b} Other studies have also demonstrated a variety of proteases in mite extracts.^{39, 41}
3. There are significant differences in the antigenicity of the recombinant mite allergens. The *Der p* I so far expressed in *E. coli* has demonstrated binding with 40% to 50% of sera containing IgE antibodies that recognize native allergen. In contrast, recombinant *Der p* II retains almost complete immune reactivity for MABs and IgE antibodies, and recombinant *Der f* II demonstrates excellent reactivity on skin testing, histamine release, and immunoblotting.^{34, 37}
4. The behavior of *Der p* I and *Der p* II in expression systems is in keeping with recent data on the stability of B cell epitopes in the native molecules. The group I allergens are readily susceptible to thermal or chemical denaturation, whereas the group II allergens are heat stable and only lose immune reactivity after reduction and alkylation.⁴² These studies complement epitope mapping studies with MAB.^{22-24, 27}
5. Polymorphisms have now been found in both group I and group II sequences, which could affect the specificity of either B cell or T cell epitopes of these molecules.
6. Tovey et al.³¹ cloned a 14 kd protein from *D. pteronyssinus*, which demonstrates no sequence homology to the group I, II, and III allergens.

Immune response

The major development has been the isolation of mite-specific T cell lines or clones by several groups, including those of O'Hehir et al.,^{32, 43} Wierenga et al.,^{44, 45} Yssel et al.,⁴⁶ Parronchi et al.,⁴⁷ and O'Brien et al.⁴⁸ T cell responses have been obtained with cells from both allergic and nonallergic individuals.⁴³⁻⁴⁵ However, in studies with purified *Der p* I or *Der p* II, significant responses were primarily observed with cells from allergic individuals.^{22, 48} Mite-allergen-specific clones have been isolated from both allergic and nonallergic individuals. More of the clones from atopic individuals supported allergen-dependent IgE production in vitro, which was interleukin-4 dependent, suggesting potential differences in the quality of T cell help.^{43-45, 47-49} Indeed, the most recent evidence (which was not available at the time of the workshop) suggests that CD4⁺ *D. pteronyssinus*-specific T cell clones from atopic donors demonstrate lymphokine secretion patterns analogous to murine "T_{H2}" cells, whereas, those from nonatopic donors are similar to "T_{H1}" cells. These data on mite-allergen-specific T cell clones provide some of the best evidence for the existence of "T_{H1}" and "T_{H2}" cells in the human.^{45, 47} Interestingly, many of the T cell clones reactive with group I or group II allergen demonstrate species specificity for either *D. pteronyssinus* or *D. farinae*. This finding is striking because human IgG and IgE antibodies to both groups demonstrate cross-reactivity, and MAbs to the group II allergens are all cross-reactive.²⁴

The question of HLA restriction to mite allergens is bound to be complex since T cells are known to be directed against several different allergens and, even if only the group I and II allergens are considered, there are almost certainly multiple T cell epitopes on each molecule. HLA restriction of T cell responses has been investigated with fibroblasts transfected with different HLA class II molecules to present mite allergen to cloned T cells. Associations between T cell proliferative responses and HLA DRB3 (DRw52), and to a lesser extent DRB1, have been observed.⁴³ Since DRw52 is in linkage disequilibrium with HLA DR3, DR5, DRw13, and DRw14, the restriction specificities are expressed in ~40% of the population. This finding could contribute to the high frequency of house dust-mite sensitivity in the general population. In spite of the somewhat permissive HLA association, there are encouraging recent studies demonstrating that a peptide that binds HLA DRw52b (derived from influenza virus) inhibits proliferative T cell responses to mite allergens in vitro.^{50, 50a} These results suggest that inhibition of T cell responses or T cell HLA interactions by peptides could form the

basis of new immunotherapeutic strategies for mite allergy.

B cell epitopes

Because allergic sensitization is a unique form of low-dose immunization, there may be distinct features of the B cell epitopes involved and immunoregulation of IgE antibody responses. The nature of the epitopes may eventually be of practical importance when altered allergens or peptides are being designed from immunotherapy. Studies with MAbs have revealed that *Der p* I has at least four epitopes, and comparisons of polyclonal antibody binding to large recombinant peptides demonstrate at least seven areas of binding distributed throughout the molecule.^{22, 23, 51} The level of binding or inhibition in these systems is, however, incomplete; therefore, the existence of a dominant epitope or binding region is possible and should be considered, considering the reported ease with which anti-idiotypic reagents can be generated.⁵² Multiple epitopes have also been defined on the group II allergens with MAb. These determinants appear to be conformational, since recombinant *Der p* II retains IgE binding, but large overlapping recombinant peptides (70 residues) demonstrate little binding.⁵³

Recommendations

Additional molecular studies are needed to clone and sequence the group III allergens, to develop improved expression systems, particularly for the group I allergens, to localize both B cell and T cell epitopes on mite allergens, and to investigate the role of antigen processing and presentation in the induction of IgE antibody responses to mite allergens. Mapping of B cell epitopes on group II allergens is currently being investigated with random fragment libraries, oligonucleotide-directed mutagenesis, and synthetic peptides. However, for these strategies to be applied to group I allergens, expression vectors capable of producing recombinant allergens that retain antibody binding activity need to be developed.

Recombinant allergens will also be increasingly needed for T cell studies. Most T cell studies have focused on clones derived from a small number of individuals. More population-based studies are needed to compare T cell responses with serologic responses to different allergens in patients with different clinical symptoms. It will also be important to identify the amino acid residues involved in recognition by T cells and antigen-presenting cells, to investigate further patterns of interleukin production by different clones (atopic versus nonatopic), and to investigate the functional significance of allergen-reactive T cells in vivo.

EVALUATION OF EXPOSURE TO MITE ALLERGENS

Standard techniques for sampling floor, furniture, or bedding dust

Sampling of reservoirs of dust within the house, measurement of specific allergens extracted from the dust, and expression of results as micrograms of group I mite allergen per gram of dust are still regarded as the best validated "index of exposure" (although microscopic determination of the number of mites per gram of dust is also valid). Thus, those recommendations of the first workshop have not changed.¹ In addition, it remains true that a modified hand-held vacuum cleaner is the easiest tool to obtain samples with the use, in general, of a 2-minute sampling of 1 m². Although total recoverable allergen is a logical measurement, it is very difficult to standardize recovery. When it is necessary to express results as micrograms or mites recovered per square meter or per tested object, it is recommended that results should also be reported as micrograms per gram or mites per gram of dust. In all cases, it is essential to specify types of vacuum cleaners, the presence or absence of filters and their pore size, and the dates and duration of collection.

Although the bedroom is generally the most important room in the house for pyroglyphid mites, other mite habitats must be considered, namely, carpets, upholstered furniture, soft toys, and clothing.⁵⁴ When avoidance measures have not been introduced, dust from mattresses or bedding is usually the best indicator of mite infestation. In some studies, interpretable results have been obtained with only one sample (e.g., from mattress) from each house. Sieving of samples has been normal practice, but it may not be essential, particularly with fine dust samples obtained from bedding, mattresses, and soft furnishings.⁵⁵ When samples include a large proportion of coarse fibers or grit, it is easier to estimate the weight of house dust in a sample after sieving. Because of the differences between dust samples from floors (more sand, grit, and coarse fibers) and from bedding and furnishings (mainly skin flakes), it is probably more valid to compare allergen levels in samples obtained from similar collection sites.

Assessment of mite exposure

There are three methods of estimating exposure to mites: mite counts, assay of mite allergens, and measurement of guanine. Mite counts have been used as an index of allergen exposure and, overall, demonstrate a good correlation with assays of group I allergen.^{22, 56, 57} The most widely used assays for measuring group I allergens are ELISA methods with species-specific MAbs to bind the allergen and labeled group-

specific antibodies for detection. The labeled secondary antibodies recognize cross-reactive epitopes on the group I allergens and may be either MAbs (clone 4C1) or affinity-purified polyclonal antibodies.^{58, 59} Since the first workshop, it has become possible to measure group II allergens, and the ratio of group I to group II allergens has been studied in dust samples, in extracts, and in airborne allergens.^{24-26, 60-62} However, more work is needed to establish whether the relationship between these two allergens is consistent. A quantitative assay for guanine has been reported to demonstrate a good correlation with assay of group I mite allergen.⁶³⁻⁶⁷ With the commercial kit, a guanine class 0 (<0.6 mg/gm of guanine) corresponds to a group I allergen content of <2 µg/gm in >80% of the samples, whereas a guanine class 2 or 3 (i.e., >2.5 mg of guanine per gram of dust) corresponds to >10 µg/gm of mite group I allergen in >90% of the dust samples. Guanine class I was found to be less informative. The quantitative assay for guanine is not generally available. A semiquantitative assay for guanine is marketed (ACAREX test) and may be a useful screening test for clinical practice.^{66, 67}

There are strong arguments for continuing to use group I allergen assays in research work and in establishing threshold values. In particular, specific allergen measurements can be compared with values for the other allergens, the assays can be standardized and compared with previous results, and the proteins being measured are allergens. Although group II allergens may be more stable than group I allergens, both allergens are stable in dust stored dry and/or in a freezer. Studies suggesting that rapid decline in group I activity can occur during extraction need confirmation. The accuracy of measurements of dust mite allergens has been made possible because of the availability of the World Health Organization-International Union of Immunological Societies international standard for *D. pteronyssinus* (National Institute for Biological Standards and Control 82/518). This preparation, by definition, contains 100,000 IU of *Der p* I and *Der p* II, which corresponds to 12.5 µg of *Der p* I and 0.5 µg of *Der p* II.^{1, 22, 24} The absolute units are much more widely used and more useful because they can be compared with results for other allergens. Similar standards (preferably with higher levels of group II allergen) are needed for *D. farinae* and *E. maynei*. The important feature of the standard is that it is stable and continues to be used as a common basis for comparison of allergen levels between laboratories.

Airborne samples

Several techniques have been described for volumetric sampling with membrane filters to capture airborne particles.^{61, 62, 68, 69} Theoretically, measurements of airborne allergen should be more representative of

exposure than assays on settled or reservoir dust. However, to date, there has been little or no data demonstrating a relationship between airborne measurements and sensitization or symptoms. The problem appears to be that concentrations of airborne allergen are generally very low and undetectable in the absence of disturbance. After disturbance, concentrations of both group I and group II allergens fall rapidly, which is in keeping with their large particle size.^{61, 62} Group II allergens also appear to fall almost as rapidly as the fecal particles carrying group I allergens.⁶² Since current data suggest that this allergen is associated with the body rather than feces, it is clear that further work needs to be done to define the particles carrying airborne group II allergens. A recent article has suggested that airborne levels of mite allergen correlated well with sensitization.^{70, 70a, 70b} However, the actual concentration of airborne allergen regarded as positive was not defined in that study, and several members of the workshop had tried very similar experiments without success. Thus, airborne measurements still have major disadvantages. In particular, they require very sensitive assays, and in the absence of disturbance, the concentrations are generally below the limits of detection. The workshop concluded that more work was necessary before any airborne measurement could be recommended as a consistent method of measuring exposure. More data are also needed comparing room sampling or personal airborne sampling with symptoms or results on reservoirs within the room.

EPIDEMIOLOGY

Before 1987, many different studies had reported an association between sensitization to dust mite allergens and asthma. During the last 3 years, several more studies of this kind have been reported. The most striking is a prospective study on a cohort of children in New Zealand followed to age 13 years. Among these children, sensitivity to both dust mite and cat dander were highly significant, independent risk factors associated with the development of asthma.⁷¹ In addition, two case-control studies in the United States found that sensitivity to mites, cats, and cockroaches were each significant risk factors for acute attacks of asthma among adults.^{72, 73} Since the first workshop, many studies have focused on quantitating allergen exposure as a risk factor for sensitization and/or asthma. Case-control studies from Berlin, San Paulo, Marseilles, and Baltimore have confirmed that asthma in mite-allergic individuals is strongly associated with exposure to $>2 \mu\text{g}$ group I allergen per gram of dust.^{2, 5, 7} In addition, a prospective study of exposure to house dust allergen (*Der p* I) and the development of asthma in children in the United Kingdom demonstrated that exposure to >10

μg of mite allergen per gram of dust in early childhood was an important determinant of development of asthma by the age of 11 years.⁶ A recent study from the same group has found that $\sim 65\%$ of children admitted to hospital for asthma in Poole, England, were both sensitized to dust mites and exposed to $>10 \mu\text{g}$ of *Der p* I per gram of dust.⁷⁴

There have been multiple studies of increasing asthma mortality worldwide during the past several years. Various causes for these increases have been discussed, including changes in house design or management and possible harmful effects of some drugs used to treat asthma.⁷⁵ Asthma deaths remain rare, approximately 4/100,000/yr, and it has been difficult to obtain specifics about risk factors for fatal asthma. However, in two areas in which mortality has been particularly common (i.e., the highlands of Papua New Guinea and the Auckland region of New Zealand), mite sensitivity and exposure are strongly associated with asthma.^{76, 77}

Although the epidemiologic evidence for a causal relationship between exposure to high levels of dust mite allergens and the development of asthma has become progressively stronger, there are many areas that still need clarification. For example:

1. Much of the data does not distinguish between sensitization and disease. Thus, it is still difficult to clearly state what concentrations of allergen exposure increase the risk of asthma in sensitized individuals. It is also not clear whether seasonal peaks in asthma admissions can be attributed to seasonal increases in mite exposure.
2. There are very inadequate data about the relationship of exposure to mites (or other indoor allergens) and severe or fatal asthma. More studies are necessary to evaluate the prevalence of sensitization and exposure among subjects with acute asthma or hospitalized subjects with asthma.
3. Many of the results imply that decreased exposure to indoor allergens would reduce the prevalence of asthma. However, this has not been adequately studied. Although it appears likely that children raised in houses without high levels of dust mites, mold, cat, or cockroach allergens will have a lower prevalence of asthma, this has not been directly demonstrated. There is increasing evidence from studies on occupational asthma that bronchial hyperreactivity induced by exposure to chemicals or antigens is irreversible in some individuals.⁷⁸ If this phenomenon also applies to inhaled allergens (such as the dust mite), it would imply that the primary objective should be to change house design and, particularly, bedroom design, so that fewer children develop asthma.

In epidemiologic studies, it is essential to define

asthma by a combination of symptoms and some objective measurements of bronchial reactivity. Unfortunately, there is still no marker for "inflammation," and none of the tests of bronchial reactivity, that is, histamine, methacholine, exercise, or cold air challenge, are fully specific. There is a need for additional studies evaluating both the effects of exposure and the techniques used for the assessment of asthma. Additional information is needed about the influence of race on specific sensitization and on the role of mites other than *Dermatophagoides* spp in sensitization and asthma. There is a need for more studies on the effects of different allergen-avoidance regimens on patients with established asthma. It would be very helpful to follow the development of asthma in a cohort of "at risk" children when they are randomly allotted to full avoidance or a control regimen for the first 10 years of life; however, this may not be feasible.

RELATIONSHIP BETWEEN EXPOSURE AND DISEASE

Several studies have assessed the relationship between levels of group I allergens in mattresses (or bedding) and sensitization to mites or allergic respiratory symptoms. Those studies have provided consistent results that led to the proposal that exposure to more than a threshold level of 2 $\mu\text{g/gm}$ will increase the risk of sensitization to mites and that exposure to $\geq 10 \mu\text{g}$ of group I allergen per gram of dust will increase the risk for overt asthmatic symptoms.⁷⁹ However, for both sensitization as well as elicitation of symptoms, it is not known whether short and heavy exposure would have more impact than long-term low-level exposure. There are still very little data on the effects of exposure at different ages on the development of disease. Although there are clear examples of adults becoming sensitized when they move to areas of high exposure, the predominant age of sensitization is still believed to be early childhood. Exposure of infants younger than 6 months to dust mites is usually low, because their mattresses are commonly covered in impervious material and because their bedding is washed frequently. However, evidence from a prospective study suggests that high-level exposure within the first 2 years of life may be highly significant for both sensitization and the risk of asthma.⁶

Particle size and allergenic load

Several studies have demonstrated that the bulk of airborne group I mite allergen is associated with the relatively "large" fecal particle, 10 to 40 μm in diameter.^{61, 62, 68, 69, 80} These data for group II allergens are less clear, but these allergens also appear to be associated with particles that fall rapidly, although the particles are probably derived from mite bodies.^{61, 62}

It is not clear what the relative role of different sized particles is in sensitization or producing symptoms. However, it is important to consider the difference between fecal particles of 20 μm in diameter carrying 0.1 to 0.2 ng of mite allergen and particles of 1 μm in diameter, for example, cat particles or nebulized droplets in which each particle carries 10^{-4} to 10^{-6} ng. Additional data are desirable about size, density, allergen concentration, and aerodynamic behavior of mite-allergen particles.

Intervention studies

Many studies have addressed the potential for reducing exposure to mites. Future studies need to address two questions that cannot be approached in a single study: The effects of reducing exposure on (1) disease development and (2) the natural history of or short-term symptoms of established disease. Since there is uncertainty about the best avoidance regimen, a careful preliminary assessment of the best method or methods to be used in the home is required. Additional convincing demonstrations that the allergen reductions that can be achieved will reduce asthmatic symptoms in sensitive individuals are needed. Such studies will have to be of sufficient duration to allow a clinically important change in disease activity to become apparent (i.e., 6 months or longer) and require a long (i.e., approximately 6 weeks) introductory period of observation and medication adjustment, without environmental intervention, to document the severity of disease. Trials should be randomized, and a placebo should be chosen so that patients and, if this is possible, physicians can remain blinded for the duration of the study. These studies will also require repeated assessments of (1) allergen exposure in the house, (2) disease activity, that is, lung function and medication requirements, and (3) airway reactivity.

ATOPIC DERMATITIS

Most patients with AD older than 7 years have high total serum IgE levels and multiple positive skin tests to inhalant allergens, including the dust mite.⁸¹⁻⁸³ These patients have been demonstrated to have high levels of IgE antibodies to group I and/or group II mite allergens and vigorous in vitro T cell responses to purified mite allergens.⁸⁴ The development of the technique of patch testing with mite allergens (sometimes referred to as the atopy patch test) has presented the possibility of investigating the role of different cells in a pathologic response to mite allergens.^{85, 86} In parallel with these studies, there has been increasing recognition that exposure to mite allergens can play an important role in the symptoms of AD and increased interest in studying the effects of dust mite avoidance as a treatment for AD.

Immunopathogenesis

The predominant cell types present in chronic dermatitis lesions are epidermal Langerhans cells, lymphocytes with a variety of phenotypic markers, and increased numbers of mast cells. Skin tests with allergens elicit an increase in wheal-and-flare responses with little or no residual response at 24 or 48 hours. The demonstration that a patch test could elicit a delay in eczematous responses provided both evidence for a role of allergen exposure in the disease *and* a model for studying the response. The eczematous response to a patch test includes eosinophils and basophils at 48 hours.⁸⁵⁻⁸⁸ Studies with serum transfer have suggested that the patch test response includes both an IgE mast cell component and a cell-mediated component.⁸⁹ The identification of Fc receptors for IgE on Langerhans cells derived from the skin of patients with AD and the associated expression of CD1 antigen provides additional evidence for a connection between IgE antibodies and cell-mediated immunity.^{87, 90-92} A positive atopy patch test to dust mites has recently been suggested to demonstrate that dust mite allergens are a cause of a patient's disease. For the test to be interpreted in this way, it would be necessary to have (1) a standardized procedure, (2) evidence that natural exposure involved comparable quantities of allergen, and (3) a demonstration that patients identified by patch testing improve when their exposure is reduced (or conversely, have symptom exacerbations when exposure to mites is increased).

The recognition of a role for house dust exposure in the pathogenesis of AD dates back to studies in the 1940s.^{93, 94} Recently, a number of studies have suggested that clinical benefit can be derived from allergen avoidance in sensitized individuals, whereas other studies have found no benefit.⁹⁵⁻⁹⁹ High levels of exposure to dust mites (≥ 100 mites per 0.1 gm of dust; ≈ 20 μg of *Der p* I per gram) have been reported to be a risk factor for severe dermatitis in patients who are sensitive to mite allergens.^{98, 100} However, there is very little other evidence relating specific levels of mite-allergen exposure to disease. Currently, desensitization treatment is not a normal part of management because allergen injections can exacerbate the disease. However, in view of the probable role of T cells, it is reasonable to ask whether peptides that only react with T cells could be an effective form of treatment for AD.

Recommendations

1. Continued studies are necessary to evaluate the specificity of IgE antibodies and T cells in patients with AD.
2. Additional studies are needed on the relationship between eczematous skin responses to patch test-

ing and the pattern or severity of clinical disease. It is possible that the atopy patch test represents a method of analyzing the mechanism of localization of allergic diseases.

3. Controlled prospective studies and/or additional well-planned case-control studies are needed to understand the relationship between exposure to indoor allergens and the development or persistence of dermatitis.
4. The most urgent clinical need is for controlled trials that define the change in mite-allergen exposure necessary to produce clinical improvement.

AVOIDANCE

Given the now conclusive evidence for the role of dust mite exposure in sensitization to dust mite allergens and the development of asthma, it is not surprising that there have been intensive efforts to develop better techniques for reducing mite exposure. Several recent studies have been able to evaluate changes achieved relative to the proposed risk levels.¹⁰¹⁻¹⁰⁴ However, there are many different recommended procedures, and in evaluating responses, it is important to be sure that the procedure itself does not interfere with the assessment of allergen present. If the cleaning procedure or the addition of acaricide changes the recovery of dust, then it may be more appropriate to calculate recovery of allergen per area (e.g., micrograms of group I allergen per square meter) or recovery from the whole object. Clear examples are water- or steam-based cleaning systems that can *reduce* the quantity of dust recovered during sampling or an acaricidal powder that remains in the carpet and therefore *increases* the amount of solids in the dust.

Physical measures

Routine cleaning with a vacuum cleaner is necessary to prevent the accumulation of allergen on the surface of carpets or furniture but is never effective at removing significant numbers of live mites.¹⁰⁵⁻¹⁰⁷ Water washing at 55°C (130°F) is effective at killing mites in bedding, will wash out allergen, and is normally recommended every 2 weeks. Hot-water washing will not denature all allergens because group II allergens require $>100^\circ\text{C}$ for complete denaturation.⁴² Although cool-water washing will remove allergen, it does not kill mites; therefore, allergen will usually reaccumulate rapidly. Liquid nitrogen will kill mites and can be applied to carpets, mattresses, or sofas. This treatment is available in the United Kingdom but has not yet been developed on a wide scale.¹⁰⁸ In areas with cold, dry winters (e.g., northern United States and Scandinavia), bedding and furnishings can be left outdoors in wintertime to freeze and dry out and thereby reduce mite populations.

Covering mattresses with zippered plastic or vapor-permeable fabrics is a very effective measure and should always be recommended.^{102, 105} If a mattress is highly infested, it should be replaced or treated before covering. Mattress covers should be inspected regularly for damage that would allow release of allergens. Removing carpets has been found to be effective in many studies, and the bedroom carpet should be removed when this is possible. At the workshop, a controlled trial of avoidance with encasings plus removal of carpets or tannic acid treatment was reported in a preliminary form.¹⁰² This study, reported at the American Academy of Allergy and Immunology meeting in March 1991, demonstrated a highly significant improvement in asthma symptoms and in bronchial reactivity, confirming the earlier study of Murray and Ferguson¹⁰⁹ that under some circumstances radical measures in the bedroom *alone* can be effective.

As the concentration of house dust mites increases with increasing indoor humidity >7 gm/kg in winter, reducing humidity may be the treatment of choice.^{110, 111} If the outdoor humidity is <5 gm/kg in winter, then increasing ventilation will allow effective removal of humidity produced by domestic activity and perspiration. However, in many parts of the world, outside humidity is >7 gm/kg, and ventilation will not solve the problem. Clearly, high microclimate humidity in carpets will also encourage mite growth. Faulty housing construction with seepage of ground water or accumulation of other water sources (e.g., condensation onto cold concrete slabs) will allow unventilated carpets to become and remain damp. Methods proposed for reducing humidity by local heating (e.g., with an electric blanket) have achieved reductions of mite numbers (40% to 80%), but the process takes weeks or months, and failure to reduce humidity by these methods could increase mite growth.¹¹² Indoor humidity reduction could be achieved by changes in national building codes; however, relevant changes in building codes would vary in different climatic regions.

Acaricides and other chemical measures

There are several chemicals that will kill dust mites in a laboratory culture, and there are many chemicals that are used to control mites in agriculture. For these chemicals to be effective in houses requires that the chemicals reach live mites. The penetration rates of currently available acaricidal sprays, solutions, foams, or powders into mattresses or carpets are not fully understood. In addition, little is known about the relevance of different types of carpets or the effect of dirt in the carpets, although it is clear that carpets

accumulate large quantities of residual dirt, which may act to "protect" mites. It is advisable to remove or replace carpets, mattresses, or sofas, which contain very high levels of mites (>1000/gm of dust) or mite allergen (>30 µg of group I allergen per gram of dust). Modern home-cleaning systems with water-based extraction procedures can achieve a very significant reduction in carpet dust/dirt. The application of suitably designed cleaning or acaricidal solutions through these machines represents an important approach that requires further consideration. However, applying "shampoos" without an adequate extraction procedure will increase the humidity of the carpet and mite growth.

Several acaricides have been marketed for use in houses, and despite varying results, significant reductions of allergen levels have been reported with pirimiphos methyl, benzyl benzoate, second or third generation pyrethroids, and natamycin (Table II).^{113-116, 119} With each preparation, a significant percentage of sites demonstrated little effect, that is, <50% reduction, and all preparations would require reapplication at intervals of 1 to 2 months. In agricultural use, mites have developed resistance to acaricides, but this has not been reported with domestic mites. Some conference participants believed that the problem of reducing mites in a thick carpet or sofa was insoluble unless the furnishings were kept dry. Most participants believed that the role of acaricides needed to be better defined and that further work was necessary on methods of application.

Tannic acid has been used traditionally as a protein-denaturing agent and was first recommended for reducing the allergenicity of house dust by Green et al.¹⁰¹ There are now several preparations available in Australia, Europe, and the United States. Tannic acid is not acaricidal, and therefore, the effect can only be temporary (i.e., weeks). A 3% (wt/vol) solution of tannic acid denatures group I allergens completely but is somewhat less effective for group II allergens. The results in carpets clearly will depend on reaching the dust reservoirs. In Australia, a preparation containing both an acaricide (benzyl alcohol), and 1% (wt/vol) tannic acid has been marketed (as DMS).¹⁰¹ However, it is important to note that the concentration of tannic acid is lower than that being used in other preparations, and as with all chemicals and acaricides, it is essential to optimize the conditions of application.

Toxicity

Evaluating the toxicity of any chemical product is complex and depends on the way it is used. Most of the chemicals proposed for killing mites are consid-

TABLE II. Carpet treatments to control dust mites and dust mite allergens

Chemical	Trade name	Mechanism	Form	Ref No.
Benzyl benzoate	Acarosan	Acaricide (used for scabies)	Powder (United States/Europe) Foam (Europe)	115
Pyrethroids	Actomite (Acardust)	Insecticide/acaricide	Pressurized canister (Europe only)	116
Pirimiphos methyl	Actellic	Insecticide/acaricide (treating grain)	Not available for domestic use	117
Natamycin	Tymasil	Antifungal (treating food)	Powder (Europe)	114
Tannic acid	Allergy control solution	Protein denaturing	Fluid (United States)	101, 118
Liquid nitrogen	—	Kills mites by freezing	Liquid gas	108
Benzyl alcohol and tannic acid	DMS spray	Acaricide and protein denaturant	Fluid (Australia)	101
Mixture of surface wetting agents and solvents	Allerex	Cleaning solution used with special vacuum cleaner	Fluid (Europe)	(Mitchell EB. In preparation)
Benzoic acid, terpinol and thymol alcohols	Paragerm	Acaricide	Fluid (Europe)	119

Ref, Reference.

ered to be safe, and there is no difficulty recommending them to treat the house of a mite-allergic patient with asthma. Recommending any application of chemicals for long-term use to *prevent* the development of sensitization would require better long-term toxicity data.

Conclusions regarding avoidance measures

It has been disappointing that no universally effective chemical treatment has been developed to treat mites. However, this is not because of the lack of effective acaricides but because of the difficulty in applying these chemicals to upholstered furniture, mattresses, or carpets. The difficulty with chemical treatments clearly focuses attention on physical measures. The current evidence that morbidity of asthma is increasing and the high prevalence of mite sensitivity among children with asthma should send a clear message to architects and regulatory agencies that houses need to be designed and maintained so as to prevent high levels of mite infestation. In the long run, it will be preferable to have polished floors, carpets that can be removed, covered mattresses, and control humidity in houses. The application of allergen-avoidance measures to patients with symptomatic asthma should be supervised both to ensure that the measures are appropriate to the sensitivity of the patient and to

avoid obsessional self-management or excessive use of chemical treatments.

CONCLUSIONS

1. Pyroglyphid mites usually account for >90% of the mite populations in houses; however, many other species, including "storage mites," may occur in houses and can become the predominant population. The term "domestic mites" should be used to include both pyroglyphid and nonpyroglyphid mites found in houses.
2. Mite sensitivity is strongly associated with asthma, and in some areas, up to 80% of children or young adults with asthma have strongly positive skin tests to mite extracts. In areas of the world with drier climates, other allergens, for example, pollens, cockroaches, and domestic animals, may replace mites.
3. Three years previously, threshold levels of mite-allergen exposure were proposed. Since that time, several case-control studies and one prospective study have confirmed the relevance of these levels. Exposure to 2 µg of group I mite allergen per gram of dust (100 mites per gram or 0.6 mg of guanine per gram) is considered to increase the risk of sensitization and bronchial hyperreactivity; exposure to 10 µg of group I mite allergen per gram

- of dust (500 mites per gram) represents a higher level of risk and increases the risk of acute attacks of asthma.
4. Exposure is best assessed by assay of allergen in reservoirs of dust, that is, mattress, bedding, carpets, and furniture. Because of the difficulty of standardizing collection, it is best to express results as micrograms (or mites) per gram of dust.
 5. Recent studies have confirmed that, in the absence of disturbance, the level of airborne mite allergen is very low, and because of this finding, it is very difficult to standardize measurements of airborne exposure. At the present time, measurements of airborne mite allergen cannot be recommended as a routine method for determining exposure. However, further studies in this area should be encouraged.
 6. Rapid progress has been made in cloning and sequencing mite allergens. This progress has allowed the production of fragments that can now be tested for reactivity with IgE antibodies, MAbs, and T cells. It may well be possible to develop peptides or modified molecules specifically reactive with T cells, which could be used for immunotherapy.
 7. Many children older than 7 years of age and most adults with AD have high or very high levels of IgE antibodies to dust mite allergens. Furthermore, application of mite allergens to their skin can induce an eczematous response. This patch test may represent a model of the chronic inflammatory response to dust mite allergen. Avoidance of mite allergens should be further investigated as a primary treatment for mite-sensitive patients.

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