

Indoor allergens and asthma: Report of the Third International Workshop

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In parallel with changes in lifestyle over the last 50 years (sedentary living in warm houses with extensive furnishing and low ventilation rates), there has been a progressive increase in the prevalence and morbidity of asthma in many parts of the world. The increase has been in perennial rather than seasonal asthma, and a large proportion of the patients are sensitized to one or more of the allergens found predominantly inside houses, that is, indoor allergens. The Third International Workshop on Indoor Allergens and Asthma was designed to discuss recent progress in basic and clinical research in this area, to formulate recommendations for allergen-specific management of asthma, and to consider future research directions. As with the two previous workshops, discussion topics included biology; allergen immunochemistry; molecular biology and immune response; epidemiology of asthma; and the role of allergen avoidance.^{1,2} Because of dramatic progress in recent years, the Third International Workshop was expanded to cover not only house dust mite allergens but also allergens from cat, dog, and cockroach, for which immunochemical and epidemiologic data are available.

Over the past 5 years there have been significant advances in several areas of research on indoor allergens, including: (1) cloning and expression of recombinant allergens,³⁻⁷ (2) analysis of T-cell responses to indoor allergens, derivation of T-cell clones, and analysis of T-cell epitope specificity and cytokine profiles,^{8,9} (3) investigation of the dose-response relationship between exposure to mite, cat, and cockroach allergens and sensitization,¹⁰⁻¹³ and (4) epidemiologic studies on indoor allergens as risk factors for the symptoms of asthma and bronchial hyperreactivity (BHR).¹⁴⁻¹⁷

Better definition of the allergens has made it possible to analyze their structure and biologic function and to define epitopes recognized by antibodies or T cells. Information obtained from those studies has provided exciting possibilities for developing new vaccines for safe and effective immunotherapy.^{9, 18, 19} Studies of T-cell responses to dust mites have confirmed the dominance of T-helper cell (T_{H2}) responses in allergic individuals.

Abbreviations used

BHR:	bronchial hyperreactivity
mAb:	monoclonal antibody (antibodies)
PBMC:	peripheral blood mononuclear cells
T _{H1} , T _{H2} :	T-helper cells

Influencing the development of these T_{H2} responses in early childhood or modifying these responses in allergic individuals should be the objective of future research on immunotherapy for asthma.²⁰

Evidence for a dose-response relationship between exposure and sensitization has been refined for both mite and other allergens. Studies in Australia, France, Germany, Japan, and the United States have confirmed that the pattern of sensitization to specific allergens reflects the mean level of allergen found in houses in the communities where the patients live.^{11, 12, 14, 16, 17, 21} Perhaps the most important finding has been the clear evidence that there are communities in which a high prevalence of asthma is associated with indoor allergens other than dust mites. In some North American cities (e.g., Atlanta, Chicago, Detroit, Cleveland, New York, and Baltimore), sensitization to allergens from German cockroach is commonly associated with asthma among low income populations living in houses containing high concentrations of cockroach allergens.^{12, 17, 22, 23} By contrast, in northern Scandinavia and in the mountain states of the United States, cats and/or dogs are the most important source of allergen related to asthma.^{11, 14, 15} In Arizona and central Australia, *Alternaria* has been reported to be the dominant allergen associated with asthma.²⁴⁻²⁶ The studies on *Alternaria* are striking because there are no other reports suggesting that allergens found outdoors have played an important role in the increases in asthma. Sensitization to pollen allergens may be significantly associated with asthma in clinic or emergency department studies. However, in population-based studies using multiple regression, sensitization to grass, ragweed, or tree pollens has not been shown to be an independent risk factor for asthma.^{16, 27, 28} Overall, the observations strongly support the view that it is specific sensitization to indoor allergens rather than generalized IgE hyperresponsiveness that is associated with perennial asthma. It is clear that understanding the immune response to the foreign antigens found in domestic houses and the subsequent inflammatory

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J Allergy Clin Immunol 1997;100:S1-S24.

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0091-6749/97 \$5.00 + 0 1/0/85691

events is central to both the investigation and treatment of asthma.

BIOLOGY

Knowledge of the biology and ecology of allergen-producing organisms is the starting point for the design, construction, and maintenance of healthy dwellings and for achieving effective allergen control regimes.

Dust mites

A pictorial key to the identification of mites found in houses (which can be referred to as domestic mites) has been published.²⁹ This includes both the house dust (or pyroglyphid) mites and storage mites, which can also contribute allergens to house dust. A database of the global distribution and abundance of mites in homes and their allergens has recently been established (D.MITE DATA).³⁰ The database enables distribution maps of clinically important species in different parts of the world to be plotted and used to determine the frequency and abundance of mite species in different countries and localities. Computer models of the relationship between outdoor climate, indoor climate, building characteristics, and dust mite population size have been designed to aid mite eradication and allergen reduction in homes.^{30, 31} There is a need for basic life-history data (developmental rates, fecundity, sensitivity to humidity) on mites, such as *Blomia tropicalis*, which have become recognized as important allergen sources in tropical and subtropical areas of the world (e.g., South and Central America, southern states of the United States, and the Far East). Mite responses to humidity are temperature dependent, and this is important when comparing dust mite populations in homes in temperate versus tropical or subtropical regions.³² Recent work has provided a basis for modeling the effects of humidity and temperature on population dynamics of dust mites.^{32, 33}

Effective control of dust mites requires understanding why some populations persist at high densities and others at low densities.³⁴⁻³⁷ Modeling of populations may have predictive value through the use of surrogates of population growth, such as measurement of the age structure of the mite population. Data on the proportions of eggs, larvae, nymphs, and adults in a population also can be used to predict whether that population is increasing or decreasing in size and how successful different control measures are likely to be.^{36, 37} The structure of mite fecal particles is maintained by a peritrophic membrane. This is important because group 1 mite allergens become airborne in the form of intact fecal particles.¹ The function of this membrane that is characteristic of arthropods is not fully understood. It has been suggested that the membrane may help to protect the hind gut from physical damage; may be relevant to coprophagia; or may act as a functional antioxidant. On the other hand, in some insects the membrane is part of the mechanism that protects the host from invasion by microorganisms in the food.³⁸

Cockroaches

Spatial analysis techniques as well as probability contours have been used to model distribution of cockroaches and their allergens in buildings in relation to changes in the distribution of food resources.³⁹ These techniques rely on the assumption that allergenic organisms are clumped, not randomly distributed, in relation to the availability of resources and have major implications for explaining the distribution of both dust mite allergens and cockroach allergens within the home. The biology of cockroaches is highly relevant to control strategies.⁴⁰ The traditional use of boric acid as a bait has been explained recently because this compound leads to progressive damage to the foregut, so that the roaches starve.⁴¹ The effect of other insecticides also may be relevant because glutathione transferase is a scavenging enzyme, and increased production is one mechanism of resistance to organophosphate insecticides.⁴² Since it also has been shown that this enzyme is a potent allergen, there is a potential for resistant organisms to produce more allergen.

Cat allergens

Histopathologic studies have demonstrated clearly that the cat allergen, Fel d 1, which is found on cat hair, is produced in cat sebaceous glands and salivary glands; in addition, large amounts of Fel d 1 have been demonstrated in cat anal glands.⁴³ Recent studies suggest that Fel d 1 production by glands in male cats is under hormonal control. Castration leads to a rapid decrease in Fel d 1 production, and injection of testosterone returns Fel d 1 to baseline levels.⁴⁴ The clinical relevance of this observation is not clear, because in the United States the great majority of symptomatic patients are exposed to cats (or dogs) that have been neutered.⁴⁵ Fel d 1 production varies strikingly in individual cats. Future research in this area could address the effects of hormonal change in female cats; the effects of age on allergen production by domestic animals; genetic control of Fel d 1 production; other methods of decreasing production of cat allergens; and the biologic role of Fel d 1.

IMMUNOCHEMISTRY AND MOLECULAR BIOLOGY

Allergen identification and nomenclature

Indoor allergens that are included in the WHO/IUIS nomenclature are listed in Table I.⁴⁶ These allergens have been purified from aqueous extracts or produced as recombinant proteins and, in most cases, nucleotide or amino acid sequences have been determined. Since the last workshop report in 1991, cloning of indoor allergens has progressed rapidly and many allergens have been identified and sequenced. Major developments include (1) cloning of allergens from mites (*Dermatophagoides* spp., *Lepidoglyphus destructor*, and *B. tropicalis*), German and American cockroaches, and dog; (2) establishing the biologic function and allergenic importance of these allergens; and (3) continued production of monoclonal

TABLE I. Structural and functional properties of indoor allergens

Source	Allergen*	MW	Function†	Sequence‡
House dust mite <i>Dermatophagoides</i> spp.	Group 1§	25 kd	Cysteine protease	cDNA
	Group 2§	14 kd	(Epididymal protein)	cDNA
	Group 3§	~30 kd	Serine protease	cDNA
	Der p 4	~60 kd	Amylase	Protein
	Group 5§	14 kd	Unknown	cDNA
	Der p 6	25 kd	Chymotrypsin	Protein
	Group 7§	22-28 kd	Unknown	cDNA
	Der p 8	26 kd	Glutathione-S-transferase	cDNA
	Der p 9	24 kd	Collagenolytic serine protease	cDNA
	Group 10§	36 kd	Tropomyosin	cDNA
	<i>Euroglyphus maynei</i>	Eur m 1	Cysteine protease	PCR
	<i>Blomia tropicalis</i>	Blo t 5§	Unknown	cDNA
	<i>Lepidoglyphus destructor</i>	Lep d 2§	(Epididymal protein)	Protein and cDNA
Mammals	<i>Felis domesticus</i>	Fel d 1§	(Uteroglobin)	PCR
	<i>Canis familiaris</i>	Can f 1§	(Taste perception)	cDNA
		Can f 2§	Calycin	cDNA
	<i>Mus musculus</i>	Mus m 1	Pheromone binding protein	cDNA
	<i>Rattus norvegicus</i>	Rat n 1§	Pheromone binding protein	cDNA
Cockroach	<i>Blattella germanica</i>	Bla g 1§	Unknown	cDNA
		Bla g 2§	Aspartic protease	cDNA
		Bla g 4	Calycin	cDNA
		Bla g 5	Glutathione transferase	cDNA
	<i>Periplaneta americana</i>	Per a 1	Unknown	cDNA
		Per a 3§	Arylphorin ²⁶⁹	cDNA

*Revised nomenclature proposed by the WHO/IUIS subcommittee.¹

†Based on sequence similarity searches of protein and nucleic acid databases. In most cases the allergen function has been confirmed by testing for biologic activity (e.g., enzyme activity). Allergens in parentheses show structural homology, but functional activity has not been confirmed or established.

‡Method given for full sequence determination, where available. However, protein sequences are incomplete; usually N-terminal or internal peptide sequences have been determined.

§Allergens for which monoclonal antibodies are available and are suitable for immunoassay purposes.

antibodies and development of immunoassays for indoor allergens.

Several candidate mite allergens for inclusion in the WHO/IUIS nomenclature were considered by the workshop. It was recommended that the 14 to 15 kd *L. destructor* allergen, formerly identified as Lep d 1, should be reclassified as Lep d 2 because this allergen showed greater than 40% sequence identity to the group 2 allergens of *Dermatophagoides* spp. and had the same molecular weight.^{47, 48} It also was recommended that three newly identified *Dermatophagoides* spp. allergens were sufficiently well characterized by sequence data, protein chemistry, and IgE antibody binding data to be added to the nomenclature: glutathione-S-transferase (Der p 8), collagenolytic serine protease (Der p 9), and tropomyosin (Der p 10 and Der f 10) (Table I).⁴⁹⁻⁵¹ The group 10 tropomyosin allergens appear to be largely responsible for IgE antibody cross-reactivity between mites and other invertebrates such as shrimp, chironomids, and cockroaches.⁵²

Molecular cloning also has identified allergens from *B. tropicalis*, *Euroglyphus maynei* and *Tyrophagous putres-*

centiae. Although a commercial RAST assay for *E. maynei* has been marketed, extracts of these species are not widely available and recombinant DNA techniques may provide the best approach to producing their allergens. A major *B. tropicalis* allergen, Blo t 5, has been identified that shows an approximate 40% amino acid sequence homology to Der p 5.^{53, 54} The prevalence of IgE antibody to Blo t 5 is 60% to 70% among patients from tropical countries (e.g., Brazil, Colombia, Singapore) who can be exposed to high levels of *B. tropicalis* in their homes.⁵⁵⁻⁵⁸ Other *B. tropicalis* allergens include a tropomyosin and several allergens of 15 to 25 kd that have been partially characterized.^{53, 54, 59, 60} Recently, two further *B. tropicalis* allergens have been cloned: a 14 kd protein that reacts with 50% of sera and has no known sequence homology and an allergen that binds to IgE antibody in 5% of sera that has homology with fatty acid-binding proteins from *Schistosoma mansoni*, mice, and humans.^{59, 60} Homologues of the *Dermatophagoides* group 1 and group 2 allergens have been amplified from *E. maynei* cDNA and the full sequence of Eur m 1 has been obtained.⁶¹ Tyr p 2, a major allergen of *T. putres-*

centiae, has been cloned and shows greater than 50% homology with the group 2 allergens from *Dermatophagoides* spp. and *L. destructor*.^{62, 63}

Sequence similarity searches have identified the biologic function of many cloned indoor allergens. The fact that several mite allergens are proteolytic enzymes secreted with the feces is well established.³⁻⁷ However, the recent observation that mite group 2 allergens show an approximately 40% sequence homology to human epididymal protein suggests that these allergens may play a role in mite reproduction and may be sex linked.⁶⁴ Two of the cockroach allergens, Bla g 2 and Bla g 5, are also enzymes (see Table I).^{65, 66} Ligand binding proteins (or calycons) were first recognized as a cause of IgE antibody responses after the cloning of cockroach allergen Bla g 4.⁶⁷ This protein family includes rodent urinary protein allergens (Mus m 1 and Rat n 1) and the dog allergens, Can f 1 and Can f 2.⁶⁸⁻⁷¹ X-ray crystallographic studies have shown that the rodent urinary allergens are pheromone-binding proteins.⁷² The dog allergens show a high degree of sequence homology (64%) with a salivary protein involved in taste reception.⁷³ Where sequence homology with known proteins has not been found, the biologic function of the allergens remains unknown, for example, mite group 5 and group 7 allergens.⁷⁴⁻⁷⁷ The function of cat allergen, Fel d 1, has not been established. Although Fel d 1 shows some homology with rabbit uteroglobin (approximately 30% in chain 1 only), it is not clear whether this homology is of structural or functional significance.⁷⁸

Standardization

The development of stable and reliable allergen standards is crucial for all studies that involve allergen measurements, principally monitoring environmental exposure in dust or airborne, assessing allergen control devices, and standardization of allergen extracts.⁷⁹ Some allergen standards that are currently in use have been developed by national or international agencies, such as the WHO/IUIS or U.S. FDA, and others have been developed by research groups or companies. Panels of monoclonal antibodies (mAb) have been produced against most of the allergens listed in Table I for use in a mAb based ELISA. These assays have become the main method for environmental allergen detection and are being used by allergen manufacturers and regulatory and government organizations for allergen standardization.^{79, 80} Most environmental studies of mite (*Dermatophagoides* spp.) exposure have relied on assays for Der p 1 or Der f 1. These assays are species specific, and usually both assays are required for measuring the total *Dermatophagoides* group 1 allergen content of dust or air samples. The introduction of group 2 allergen assays has provided another marker of mite allergen exposure that may be particularly useful in allergen avoidance studies because the group 2 allergens are more resistant to heating and denaturation than their group 1 counterparts and are more cross-reactive within the genus.^{81, 82} Antibody binding to group 1 allergens can be masked by

some wheat components, resulting in an underestimation of the mite content of bakery dust (a problem not observed for group 2 allergen).⁸³

Immunoassays for mite group 5 allergens (Blo t 5 and Der p 5) have been developed recently and may be useful for assessing allergen exposure and cross-reactivity between *Blomia* and *Dermatophagoides* species.^{84, 85} Assays for Fel d 1, Can f 1, Bla g 1, and Bla g 2 have proved effective in monitoring exposure to animal dander and cockroach allergens in both dust and air samples.^{5, 6, 11, 12, 17, 80, 86-88} An assay for rat urine allergen also has been developed but has not yet been tested for environmental studies.⁸⁹ Measuring fungal allergen exposure in dust samples continues to be a problem. Although several laboratories have produced mAb to fungi (e.g., *Aspergillus* and *Alternaria*), these assays have been unsuitable for environmental allergen measurements either because they lack sensitivity or because the allergen being measured is not found in fungal spores.⁹⁰ The development of reliable assays for fungal allergens, or other fungal metabolites that correlate with exposure, is a high priority for further studies on the role of indoor fungi in causing asthma.

Almost all the standards currently in use are allergen extracts with a given concentration of specific allergen, either in arbitrary units or in absolute values (nanograms or micrograms of protein). The use of absolute values is preferred because it allows direct comparison between values for different allergens and has greater scientific merit. However, with the development of improved allergen purification techniques and the advent of recombinant allergens, discrepancies have been found between the reported absolute levels of given allergens in extracts and allergen standards. For example, previous workshops have endorsed the WHO/IUIS *D. pteronyssinus* standard as containing 12.5 µg per ampoule Der p 1; however, two recent studies obtained a value of 5.0 µg per ampoule, suggesting that the 12.5 µg value may be an overestimate.^{91, 92} Similarly, reported estimates of an FDA unit of Fel d 1 range from 2 to 4 µg of protein.^{93, 94}

To achieve a consensus over allergen measurements and for these studies to be directly comparable, it is vital that allergen measurements are expressed against a single common standard. In view of the uncertainty of the mass estimates for allergen units, it is strongly recommended to include the conversion factor adopted (nanogram per International Unit, or per Allergy Unit) in scientific publications. The current estimates are 125 pg/IU for Der p 1 (range, 40 to 125), 1 pg/IU for Der p 2 (range, 0.5 to 5), 2000 ng/IU for Fel d 1 (range, 1000 to 4000), and 1 ng/IU for Can f 1 (range, 0.3 to 3). With respect to Der p 1, it is important to note that the current value (125 pg/IU or 12.5 µg per ampoule) has been used in many epidemiologic studies and in establishing threshold values for mite exposure leading to sensitization (2 µg of group 1 allergen per gram of dust). In order not to compromise the comparability of epidemiologic and interventional studies, it is recommended

to continue to use the 12.5 µg per ampoule value. Clearly, the availability of recombinant allergens will in the future make it possible to use purified recombinant proteins as absolute standards and will largely circumvent these problems. Recombinant Der p 2 can be produced in sufficient quantities to prepare 1000 vials of a 100 µg per vial standard, and the workshop endorsed a program to prepare and distribute recombinant allergen standards for research purposes. Such standards would comply with WHO and U.S. FDA guidelines concerning recombinant proteins.

MOLECULAR BIOLOGY

The first complete amino acid sequence of an indoor allergen was published in 1988.⁹⁵ Since then, cDNA cloning and PCR-based sequencing techniques have successfully defined the structures of major allergens from mites, cat, dog, and cockroach. Where do we go from here? Molecular biology is moving into new phases, encompassing expression of recombinant allergens, analysis of sequence polymorphisms for individual allergens, comparison of allergen expression in related species, and determination of three-dimensional structure. High-level expression of recombinant allergens is crucial to obtain sufficient quantities of protein for structural and immunologic studies. A number of bacterial and yeast expression vectors have been used successfully to produce recombinant indoor allergens, including pGEX and pET (in *Escherichia coli*) and pSAY or *Pichia pastoris* (in yeast).^{53, 62, 96, 97} In some cases, the immunoreactivity of the recombinant allergens, as assessed by serologic IgE antibody assays and in vivo skin testing, is very good. Thus recombinant mite group 2, 5, and 7 allergens and the cockroach allergens Bla g 4 and Bla g 5 react strongly with IgE antibodies.^{53, 62, 66, 67, 74, 75, 97, 98} In other cases, the natural allergen has not yet been purified (e.g., Blo t 5, Der p 5, Bla g 4) and the recombinant allergens alone appear to be effective markers of IgE antibody responses. Optimal expression systems for recombinant allergen production must be established. There have been problems in expressing group 1 mite allergens with good immunoreactivity with the use of either bacterial or yeast vectors.⁹⁹ Der p 1 with full enzymic activity has been obtained from proenzymes expressed in baculovirus, but the yield needs to be improved to make this a practicable system.¹⁰⁰ Similar problems in allergen expression have been observed with the cockroach aspartic protease allergen Bla g 2, which has five potential disulfide bonds and has not been expressed in immunoreactive form.⁶⁵ Fel d 1 chains 1 and 2 expressed in bacteria (with His tags) bind IgE antibody, though not as well as the natural allergen. The chains can be refolded into an immunoreactive molecule, but the feasibility of doing this on a large scale has not been established.¹⁰¹⁻¹⁰³

The workshop recommended that the immunologic reactivity of recombinant allergens should be established by several techniques including skin tests, serum IgE antibody assays, and histamine release and that where

possible, the activity of recombinant and natural allergens should be quantitatively compared. The availability of a large number of recombinant allergens now makes it feasible to consider whether these reagents could be used for diagnostic and therapeutic purposes, as an alternative to conventional allergen extracts. For diagnostic purposes, this would involve formulating a cocktail of two to four recombinant allergens that would have the same sensitivity and specificity as the conventional extract in both in vivo and in vitro tests. Candidates for dust mite would be the group 1, 2, 5, and 7 allergens and for cockroach, Bla g 1, 2, 4, and 5. Clinical studies of skin test responses to recombinant mite and cockroach allergens are now underway in several centers across the world and should establish the feasibility of this approach, the numbers of allergens required to form a successful cocktail, and the extent to which further expression of other allergens is required. Once the diagnostic specificity of recombinant allergens is established, it then would be possible to carry out trials to assess their efficacy in immunotherapy.

Sequence polymorphisms and structure-function relationships

Naturally occurring sequence polymorphisms have been found for the mite group 1, 2, and 3 allergens after PCR-based sequencing of genomic DNA from mites (*Dermatophagoides* spp.) obtained from different parts of the world. These polymorphic allergen variants, or isoforms, usually show between three and six amino acid residue differences, which can affect both antibody binding and T-cell reactivity.^{48, 96, 104-107} Variability in mAb binding to group 2 allergens has been demonstrated and needs to be taken into account when using mAb in immunoassays, especially if recombinant group 2 is to be used as a reference standard (the reference preparation should be shown to have broad mAb specificity).^{96, 107} The quantitative distribution of polymorphic variants in wild or cultured mite populations has not been established. In wild populations, multiple variants are likely to be produced and, while mAb may underestimate certain isoforms, it seems unlikely that these effects will significantly influence measures of environmental allergen exposure provided that the mAb are carefully selected. Recent evidence suggests that the amino acid substitutions in isoforms of Der p 1, Eur m 1, and group 2 allergens affect proliferative T-cell responses in approximately 20% to 30% of patients allergic to mites.¹⁰⁶ Further investigation of the spectrum of isoform sequences may help design allergen peptides with optimal reactivity for use in immunotherapy studies.

Computer modeling techniques have been used recently to determine a detailed three-dimensional structure of Der p 1 on the basis of its homology with papain, and similar techniques have been applied to model structures for Bla g 4 on the basis of its homology with other calycons, including rodent urinary allergens.^{67, 72, 108} Obtaining the tertiary structure of these molecules will allow more accurate prediction of surface

exposed residues, which form potential antigenic sites, and will allow targeting of these amino acids for site-directed mutagenesis.¹⁹ The tertiary structure of Der p 2 is now being resolved by nuclear magnetic resonance spectroscopy and x-ray crystallography. It is anticipated that as high-level expression systems are developed for other allergens, an increasing number of tertiary structures will be determined, and these structures may shed some light on the function of allergens that are as yet unknown. Structural information will also make it possible to design molecules that lack biologic function (e.g., enzymic or ligand binding activity) and to investigate the extent to which biologic activity influences allergenicity. It has been proposed that the enzymic function of several mite allergens enhances their ability to induce IgE responses by increasing mucosal permeability.^{4, 109} In support of this hypothesis, two recent studies have shown that the Der p 1 cysteine protease can cleave CD23 from B-cell lines, suggesting a possible mechanism of enhancing IgE, because sCD23 fragments can upregulate IgE production.^{110, 111}

Recommendations

Recombinant allergens with comparable immunoreactivity to the natural allergen should be produced and evaluated for allergen standardization, diagnostic testing, and immunotherapy. These allergens should be tested either singly or in combination with other allergens from the same source, and the activity of allergen isoforms also should be compared. In cases in which current recombinant allergens are unsuitable, improved expression systems are needed to produce functionally and immunologically reactive proteins and to increase allergen yields.

Where possible, the tertiary structures of major allergens should be determined. This will allow localization of residues involved in IgE antibody and mAb binding (e.g., by targeting surface exposed residues for mutagenesis) and of residues that contribute to the biologic function of the molecule.

IMMUNE RESPONSE AND IMMUNOTHERAPY

Mite allergens

IgE antibody prevalence. An interesting aspect of the molecular cloning studies is that the group 1 through 10 allergens now cloned account for most of the IgE antibody binding bands previously identified by immunoblotting or crossed radioimmuno-electrophoresis. A comprehensive immunoblotting study showed a high frequency of IgE binding bands at 15, 16, 25, 26, 30/32, 54/56, and 90 to 100 kD, and the allergens now characterized would account for these reactivities.¹¹² Sera from most patients recognize between two and six allergens on immunoblotting, though a significant number clearly recognize more than 10.¹¹³ Blotting data are not quantitative, and some allergens (e.g., Der p 3, Der p 7) may show multiple bands on sodium dodecylsulfate-polyacrylamide gel electrophoresis, which complicates estimates of antibody prevalence. These bands may repre-

sent different molecular forms of the same allergen (e.g., Der p 7) or enzymatic breakdown products of the allergen (e.g., Der p 3).^{76, 114}

The ability to use allergens as markers of environmental mite exposure is also relevant to the immune response. Both group 1 and group 2 allergens can be detected readily in house dust samples and can occur at high concentrations, whereas the levels of other mite allergens (e.g., group 5) appear to be significantly lower (which is presumably the reason for the lower prevalence of sensitization to these allergens). Data on allergens other than group 1 and 2 are still being accumulated, and their relative importance is being established. Approximately 50% of sera react to Der p 7, and the level of IgE anti-Der p 7 antibody can be higher than that to Der p 2. Patients often have high levels of antibody to both allergens.¹¹⁴ Comparisons of IgE antibody binding to different purified allergens are needed, and these studies should include sera from children and adults as well as sera from patients in different geographic areas and occupations. A chimeric mouse/human anti-Der p 2 mAb has recently been constructed, which can be used for absolute quantitation of IgE antibodies to Der p 2 or by extrapolation to other purified allergens.¹¹⁵

Given the large panel of defined mite allergens, it is pertinent to ask which are the most important in terms of IgE reactivity and how this should be defined. The terms "major" and "minor" allergens have been defined in the past on the basis of the prevalence of serum IgE antibodies in selected groups of patients allergic to mites. However, the sensitivity of IgE detection systems has increased, and most of the mite allergens listed in Table I can be shown to react with IgE antibodies in more than 50% of sera. For example, a very high prevalence of IgE antibodies to Der p 3 has been shown in some studies.⁵⁰ Additional criteria should be used to assess allergenic importance, including measuring the percentage of allergen-specific IgE antibodies (in terms of IgE antibody to mite extract) and the extent to which individual allergens contribute to the allergenic activity of mite extract. These assessments can be made by absorbing sera with purified allergen or by absorbing the extract with the use of monospecific antibody (either monoclonal or polyclonal). It has been proposed that major allergens should be regarded as those components to which more than 10% of the IgE antibodies of more than 50% of the patients react. On the basis of absorption studies, only group 1 and group 2 allergens would be considered "major" allergens for *Dermatophagoides* spp. The prevalence of IgE antibodies to group 1 and 2 allergens is greater than 80%, and immunoabsorption studies have shown that greater than 50% of the IgE antibody to mite can be directed against these allergens.⁹² A recent study has also shown that the magnitude of IgE responses to the group 1 and 2 allergens is greater than to Der p 6 or Der p 9.⁵⁰

Because of the confusion between criteria based on the prevalence of sensitization and those based on

absorption studies, combined with the fact that assays for IgE antibodies have increased in sensitivity, the term major allergen cannot have absolute criteria. Two different criteria should be used in evaluating purified/defined allergens:

(1) An allergen can receive WHO/IUIS nomenclature once it is defined (i.e., physical properties and sequence) and it has been demonstrated that at least 5% of allergic individuals react to it (skin tests or IgE antibodies).

(2) Judging the importance of an allergen should take several features into accounts:

- The prevalence of sensitization relative to other proteins from that source.
 - The proportion of IgE antibody to the source that is specific for that allergen, estimated either by absorption studies or by quantitative immunoassays or IgE antibody binding.
 - The quantity of the allergen in house dust.
- Additional factors that are relevant to evaluating defined allergens include:
- T-cell responses in vitro.
 - The availability and immunogenicity of recombinant allergen.

While there are no absolute criteria for whether an allergen should be judged as important (or major), the question is relevant to identifying which allergens should be present in an allergen extract or in a mixture of recombinant proteins used for diagnosis or immunotherapy.

T-cell responses. Studies of T-cell recognition of mite allergens have compared proliferative responses, epitope specificity, and phenotype of cells derived from allergic or nonallergic donors, as well as from allergic patients with different clinical symptoms. These studies have used peripheral blood mononuclear cells (PBMC), T-cell lines, or clones and examined both cell surface markers and patterns of cytokine production. Since the previous workshop, there have been significant advances in these areas, particularly in defining the epitope specificity and phenotype of T-cell responses to purified allergens (Der p 1 and Der p 2). Lymphocytes from most donors allergic to mites make proliferative responses to Der p 1 and Der p 2, which show a good quantitative correlation.^{116, 117} T-cell lines and clones isolated from allergic donors are usually of the T_{H2} phenotype and produce IL-2, IL-4, and IL-5.^{9, 118, 119} The frequency and magnitude of T-cell responses is lower in nonallergic donors (though this depends to some extent on culture conditions), and T-cell clones isolated from non-allergic donors are usually T_{H1} (i.e., produce IL-2 and interferon- γ but little IL-4 or IL-5).⁹ Epitope analyses have relied on stimulating PBMC or T-cell clones with synthetic peptides or fusion peptides. Studies from several laboratories have identified peptides from Der p 1 or Der p 2 that induce T-cell proliferation (reviewed in Reference 9). For Der p 1, the evidence suggests clustering of T-cell sites in the

central region of the molecule, spanning residues 95 to 130. The sites on Der p 2 that stimulate T cells are distributed throughout the molecule, and peptides 61 to 86, 78 to 104, or 105 to 129 have been identified as being important, with a tendency for the T-cell epitopes to be located toward the C-terminus.¹²⁰⁻¹²² To date, most T-cell epitopes have been identified through the use of cells derived from allergic donors. HLA class II restriction and T-cell receptor usage have been compared in several studies. No clear HLA association with T-cell responses has been observed, except that the restricting elements can be DR, DP, or DQ. One study found an association between the magnitude of the T-cell response to Der p 2 and HLA DQ7.¹²³ There does not appear to be a strong correlation between T-cell responses to mite allergens and T-cell receptor usage, although a preference for V β 3 has been reported.¹²⁴

It is difficult to make direct comparisons of the T-cell studies because they often involve PBMC from small groups of patients or clones derived from a few patients. Generally, data on epitope specificity have been obtained from clones derived from only one or two individuals, and the extent to which these epitopes are relevant to a general population of patients allergic to mites is unclear. There have been few studies on the responses of polyclonal T cells from a large number of patients to synthetic peptides, and there are no data on allergens other than Der p 1 and Der p 2. In view of the importance of this area in developing new strategies of immunotherapy, more systematic studies of T-cell responsiveness to allergens/peptides are needed involving large and well-defined patient populations.

Immune response in early childhood

A prospective study of children born to at least one allergic parent showed that IgG and IgE antibody responses to mite allergen (Der p 1) developed in the second and third years of life.¹²⁵ This study used an antigen-binding radioimmunoassay to measure antibody responses. Subsequently, IgG antibody responses to mite extract were detected in atopic and nonatopic children as early as 3 months of age by ELISA, and this technique also detected IgG antibodies in more than 80% of nonatopic adults (as compared with approximately 20% using antigen-binding techniques).^{126, 127} While these techniques give differences in estimated prevalence of antibody responses, the evidence suggests that antibodies to mite allergens can be detected during the first year of life. Recent studies have also shown that cord blood T cells proliferate when cultured with mite extracts and, in some subjects, with purified Der p 1 or Der p 2.^{128, 129} The significance of results obtained with mite extract are difficult to interpret because of potential nonspecific effects. However, the observation that these responses can be obtained with purified allergens has aroused interest in whether sensitization to inhaled allergens (as well as food allergens) can occur in utero. The interpretation of these data is controversial, in part because it is

difficult to envision how a fetus could become sensitized to the minute amounts of allergen inhaled by the mother and also because some of the results have been obtained with the use of serum-free medium. Almost 100% of nonatopic individuals can be shown to have T-cell responses to mite allergens with the use of this type of medium (e.g., AIM V), a prevalence that is much higher than had previously been obtained with serum-containing media.^{130, 131} At present, the evidence does not resolve whether the T-cell responses seen in serum-free media are allergen specific or when these cells develop during the course of the immune response.¹²⁸⁻¹³² It is also unclear whether these T-cell responses occur as a consequence of mite exposure. In some studies the response of the fetal cells is dependent on maternal exposure. However, responses to mite allergens in vitro have been observed with the cord blood of Swedish infants whose mothers were exposed to less than 0.4 µg/g group I allergen.¹³³ Studies of the T-cell responses in early childhood are of great importance in establishing at what age attempts to intervene in the sensitization process should be introduced (either by allergen avoidance or by active immunization).

Cat and dog allergens

The major cat allergen, Fel d 1, is a heterodimer, comprising chain 1 (70 amino acids) and chain 2 (90/92 amino acids). The sequences were obtained by PCR and protein sequencing and the genomic DNA sequence of Fel d 1 has subsequently been determined.^{78, 134} Approximately 90% of patients allergic to cats make IgG and IgE antibodies to Fel d 1, which can account for 50% of the IgE antibodies to cat by RAST and 60% of histamine releasing activity. Absorption of Fel d 1 removes 60% to 95% of the allergenic activity of cat dander extracts.^{135, 136} Consequently, Fel d 1 has been an excellent marker of the immune response to cat allergens and has been used to monitor changes in antibody responses and T-cell responses during immunotherapy. However, at least eight other cat allergens have been identified in crossed radio immunoelectrophoresis (CRIE)/immunoblotting studies, including a 30 kd allergen that has been partially characterized.¹³⁷ Quantitative data are needed to compare the prevalence of IgE antibodies with these allergens.

Reduction and alkylation of purified Fel d 1 eliminates most of the antigenicity, although more than 50% of the IgE-binding activity can be detected with isolated recombinant chains.^{102, 103} Binding of IgE antibodies to synthetic peptides of Fel d 1 has been compared, with peptides 25-38 (chain 1) and 15-28 (chain 2) showing significant activity (46% and 28% binding, respectively). The affinity of IgE antibody binding to these peptides appears to be approximately 100-fold lower than to the natural allergen.^{101, 138}

Although there are as yet few published reports of T-cell responses to Fel d 1, T-cell reactivity to Fel d 1 has been investigated as part of attempts to develop a peptide-based vaccine for cat allergy.^{18, 139} Two peptides, corresponding to residues 7-33 and 29-55 of chain 1,

have been reported to stimulate peripheral blood T cells from both allergic and nonallergic individuals.^{18, 140} More recently, shorter T-cell epitopes on both chain 1 and chain 2 have been identified by T-cell cloning studies with cells derived from a small group of donors allergic to cats.¹⁴¹ In that study, only 30% of the T-cell clones that proliferated in culture with cat extract were specific for Fel d 1 peptides, which suggests that other allergens need to be studied.

There have been only limited numbers of studies of the immune response to purified dog allergens (Can f 1 and Can f 2), which in part reflects the fact that these allergens have only recently been identified and cloned.⁷⁰ Serologic estimates of the prevalence of IgE antibody to these allergens are 70% for Can f 1 and 23% for Can f 2.⁷¹ There are no published data on T-cell responses to Can f 1 and Can f 2. However, there are some data to suggest that dog is a less potent source of allergens than cat; most patients allergic to dogs are also sensitive to cats, but the reverse is not true, and patients who are solely sensitive to cats are common.

Cockroach allergens

Although there have been initial studies to establish the prevalence of IgE antibodies to the cloned cockroach allergens listed in Table I, the immune responses to these allergens have not been extensively studied. Serologic comparisons of IgE antibodies to Bla g 1, 2, 4, and 5 using more than 70 sera suggest that measuring IgE antibodies to this cocktail of allergens will demonstrate approximately 95% of sensitization to *B. germanica*, and preliminary studies have shown that mAb affinity purified Bla g 2 and recombinant Bla g 4 can elicit T-cell responses in patients with asthma who are allergic to cockroaches.¹⁴²

New therapies and prophylaxis

Knowledge of the immune response to indoor allergens and their structure presents an opportunity to develop more rational strategies for the immunotherapy of allergic diseases, and several new approaches are being investigated. The production of recombinant allergen vaccines including several allergens from a source is one possibility, but these preparations would still have the potential to generate adverse reactions if they used unmodified allergen molecules. Peptide-based vaccines are attractive because the peptides themselves would be expected to have little reactivity with IgE antibodies. Phase III clinical trials of T-cell peptides from Fel d 1 showed that administration of 75 to 750 µg Fel d 1 chain 1 peptides reduced symptom scores in patients with mild asthma after allergen challenge in a cat room and suggest that such approaches can be effective.¹⁸ Related approaches include the use of modified peptides or production of allergen variants that lack IgE antibody binding activity.¹⁹ Studies with Der p 2 variants have shown that substitution of cysteine residues can generate variants that have reduced reactivity with IgE antibody but retain T-cell epitopes. This approach offers the

possibility of using full-length allergen molecules (containing the majority of T-cell epitopes) for immunotherapy while reducing the risk of anaphylactic reactions. Strategies based on using recombinant allergens or peptides assume that downregulation of the IgE responses to a limited number of allergens or peptides would have concomitant effects on reactivity to other allergens from the same source. Evidence for this has been obtained from animal studies that show that inhibition of antibody and cellular responses to whole allergens can be achieved with peptides containing only one epitope.^{139, 143, 144}

Influencing pathways of T-cell differentiation from T_{H2} to T_{H1} is another potential approach to immunotherapy. This could be achieved by the use of different methods of antigen presentation or by the use of naked DNA vaccines.¹⁴⁵⁻¹⁴⁷ Recent studies have shown that a naked DNA Der p 5 vaccine reduced airway hyperreactivity and IgE responsiveness in rats and was associated with the appearance of CD8+ T cells.¹⁴⁸ Naked DNA vaccines elicit T_{H1} responses and can switch preexisting T_{H2} responses to T_{H1}. It also may be possible to use other adjuvants to potentiate T_{H1}-like responses to allergens in early life, which would be boosted after natural allergen exposure and lead to "suppression" of T_{H2} responses.²⁰ The effects of conventional immunotherapy on allergen-specific cytokine production have been investigated and have shown reductions in IL-4 production, with variable results on interferon- γ and other cytokines.^{149, 150} Attention is increasingly being focused on the possible use of immunologic intervention in early life, before allergic disease has developed, as a classic immunoprophylactic approach. Identification and targeting of at-risk individuals (e.g., children born to an atopic parent) are essential in designing studies to investigate whether such a strategy would be effective.

Recommendations

Given the large number of allergens that have now been cloned, it is important to establish the immunologic and clinical significance of individual allergens to assess their importance. Quantitative comparisons of both antibody and T-cell responses to mite, cat, and cockroach allergens are necessary to evaluate their potential for use in immunotherapy.

T-cell studies should be directed at large and well-defined clinical populations and should investigate epitopes recognized by polyclonal T-cell populations as well as T-cell clones and lines. To reduce nonspecific effects, these studies should use purified or recombinant allergens. The role and importance of specific types of antigen-presenting cells in allergen-specific T-cell responses should be a focus of future studies.

Most cellular studies have focused on adult populations. In view of the importance of early childhood exposure in allergen sensitization, studies should be directed toward investigating the immune response in infancy. This will require technical improvements in isolating relevant cell populations from babies and

younger children. The question of in utero sensitization needs to be further evaluated by investigating potential confounders (e.g., the titer and specificity of maternal IgG antibody) and carrying out studies on families living in areas of high or low allergen exposure.

ASSESSMENT OF EXPOSURE

The published data on the relationship between exposure to dust mite allergens and sensitization to these proteins are based on measurement of allergen concentration in dust ($\mu\text{g/g}$). The dust samples were obtained from reservoirs within the house, for example, bedding, mattresses, carpets, and sofas. From these results it is clear that expressing results as micrograms of allergen per gram of dust is still the most consistent method available.^{1, 2} An external quality control program for the measurement of Der p 1 has been established by the Wellington Asthma Research Group (R. Siebers and J. Crane, Wellington School of Medicine, PO Box 7343, Wellington South, New Zealand). Preliminary results demonstrated a sixfold to sevenfold variability in Der p 1 results worldwide, which in part may be due to Der p 1 extraction temperature differences.¹⁵¹

In many studies, assay of allergens in dust from bedding has provided the informative data and can be taken as an index of exposure to mite allergens. For mite avoidance studies, expression of allergen as micrograms per gram of dust may fail to express the change in exposure after certain procedures are done (e.g., covering mattresses).^{152, 153} In studies of this kind, measurements of allergen per unit area (m^2) should be included (this depends on measuring both the concentration of allergen and the total weight of dust collected). In keeping with the results of the first workshop, it remains true that most investigators have been unable to detect airborne mite group 1 and 2 allergens in the absence of experimental or household disturbance. Very low levels have been reported in houses with normal levels of domestic activities (20 to 60 pg Der p 1/ m^3). However, these levels are below the detection limit for most assays and it seems that there is no reproducible method to assess mite airborne exposure suitable for studying multiple houses. The Petri dish method of leaving dishes out for 2 weeks represents an interesting approach, but more studies are need to validate the technique.

Several studies have shown that 20% to 30% of airborne Fel d 1 is associated with particles of apparent aerodynamic size less than 5 μm , and in keeping with this it is possible to measure Fel d 1 in the absence of disturbance. Measuring airborne cat allergen could be considered as a routine approach to assess the relationship between the airborne allergen sensitization, and symptoms. These measurements are also dependent on multiple variables, particularly disturbance and ventilation.^{154, 155} In all epidemiologic studies to date, exposure to cat allergen has been reported as the concentration of Fel d 1 in micrograms per gram of reservoir dust. On the other hand, it should be recognized that in individual houses, comparisons of exposure levels and sensitization

to cat allergen have not shown correlations as good as those reported between mite allergen exposure and sensitization.^{11, 87} Dog allergen Can f 1 has aerodynamic characteristics similar to Fel d 1.⁸⁸ In contrast, two groups have reported that cockroach allergens (Bla g 1 and Bla g 2), like mite allergens, are only airborne during disturbance of dust in the house.^{156, 157} In general, the highest concentration of cockroach allergen has been found in the kitchen, and the best indicator of cockroach exposure is micrograms per gram of Bla g 1 or Bla g 2 in dust obtained from reservoirs in the house.^{12, 17, 22, 23, 157, 158}

Recommendations

In many studies, the concentration of cat allergen in individual houses does not define the risk of sensitization. This may be because cat allergen becomes widespread in the community (i.e., in schools and day care centers; also in houses that do not have a cat) or because the concentration of Fel d 1 in reservoirs within the house is not a good index of the quantity of allergen inhaled. Future studies should investigate this problem by measuring airborne cat (and or dog) allergens in houses, schools, and day care centers in relation to sensitization of children in a community. The question is whether sensitization of children in a community relates to exposure in individual houses or to total exposure.

Questions about exposure assessment remain important in evaluating avoidance studies because some measures can decrease the quantity of house dust without necessarily changing concentrations of allergen. Other measures may change the relationship between reservoir dust and the quantity becoming airborne. Avoidance protocols should be evaluated where possible by using measurements of reservoir dust concentration and quantity and by airborne measurements during disturbance.

Alternative methods of exposure assessment must be considered, including sampling air in houses during moderate disturbance, provided that the disturbance procedure can be standardized, and imaginative approaches to personal sampling and sampling close to bedding or furniture, which may require prolonged sampling at low flow rates.

EPIDEMIOLOGY

The strongest evidence for the causal role of allergen exposure in perennial asthma has come from studies on dust mite allergens and dust mite sensitization.^{1, 2} The criteria for demonstrating causality between an environmental exposure and a noninfectious disease were first proposed by Bradford-Hill in 1965.¹⁵⁹ Review of the evidence relative to these criteria leads to the conclusion that dust mites must be a major cause of asthma.^{2, 160} The experimental evidence from bronchial provocation and avoidance studies is clear, as is the consistency of the association between sensitization to indoor allergens and asthma. Some of the criteria proposed for causality are more difficult to establish but nonetheless important; the most obvious of these are temporality and dose

response.^{10, 125, 160, 161} Over the last 10 years, evidence has accumulated that exposure to mite allergens has a critical role in the development of bronchial hyperreactivity. However, the argument has become more complicated because of studies that have associated other indoor allergens with a high prevalence of asthma. Furthermore, some cross-sectional studies have not found a dose-response relationship between exposure and the severity of symptoms of asthma (Table II).

Exposure and sensitization

Demonstration of a dose-response relationship between dust mite allergen exposure and sensitization has proved to be relatively straightforward,^{1, 2} but this has not been simple for asthma symptoms.¹⁶² The power to demonstrate an effect relies on sampling from a wide range of exposures that for a nonseasonal allergen are unlikely to occur in any single community.¹⁶³⁻¹⁶⁶ Over the last 3 years, studies from four different groups have confirmed (1) dose-response relationship between exposure to mite allergens and sensitization to these allergens,^{10, 167, 168} (2) the dominance of dust mite sensitization as a risk factor for asthma in communities with high levels of exposure,^{24, 28} and (3) clear evidence about other communities with low mite exposure in which other indoor or outdoor allergens dominate sensitization.^{11, 14, 15} In Australia, the risk of house dust mite-sensitized children having current asthma doubled with every doubling of Der p 1 levels between 0.7 and 50 $\mu\text{g/g}$ dust.¹⁶⁸ In the United States, with the use of adjusted odds ratios, sensitization to dust mite allergens has been shown to be the single strongest risk factor for asthma among middle school children on the East Coast.²⁸ In the mountains of New Mexico, sensitization to cat and dog allergens was very strongly associated with symptomatic BHR.^{11, 87} Similarly, in areas of Australia and Arizona where mite levels were low, sensitization to *Alternaria* and/or cat allergens was more important.^{24, 25} In New Zealand, all the data have related asthma to sensitization to mite allergens, reflecting the very high humidity levels.^{27, 153, 169} A strong confirmation of the dose-response relationship between mite allergen concentration in bedding and the risk of sensitization has been reported from Germany (References 10 and 167; also see 160, 161, 163, and 170). These authors also demonstrated that the "threshold" level for atopic children was approximately 2 μg group 1 allergen/gram, while the comparable level for nonatopic children was approximately 50 $\mu\text{g/g}$.¹⁶⁷

Sensitization and asthma

The evidence for an association between sensitization to dust mite allergens and asthma was extensively reviewed in the first and second workshop reports.^{1, 2} In the last few years, several groups have presented the evidence as adjusted odds ratios. Analyses of this kind have demonstrated that mite sensitization is the major independent risk factor for asthma in New Zealand,^{27, 169} coastal Australia,¹⁶⁸ Florida,¹⁷¹ and central

TABLE II. Sensitization and exposure to indoor allergens as a risk factor for asthma, 1989-1995

Authors	Geographic location	Allergens	Odds ratio/Significance
<i>Population studies</i>			
Arruda et al. 1991	Sao Paulo, Brazil	Mite	$p < 0.001^*$
Peat et al. 1991-1995	Coastal and inland Australia	Mite, "animal dander"	2.7-20.9*; 2.4-4*
Lau et al. 1989	Germany	Mite	5-11‡
Price et al. 1990	London, UK	Mite	$p < 0.01‡$
Wickman et al. 1991; 1993	Stockholm, Sweden	Mite	4.9*; 25.7†
Kuehr et al. 1995; 1992	Southwestern Germany	Mite, "animal dander"	2.2-4*; 2.6-3.6*; 2.8†
Custovic et al. 1995	Manchester, UK	Mite	$p < 0.01†$
Rosenstreich et al.	Cities Northeast USA	Cockroach	$p < 0.001†$
<i>High altitude studies</i>			
Sporik 1995 et al., Ingram et al. 1995 }	Los Alamos, N.M.	Cat and dog	6.2†; $p < 0.001^*$
Charpin et al. 1991; 1988	Martigues and Briancon, France	Mite	$p < 0.02^*$
<i>Emergency department studies</i>			
Gelber et al. 1993	Wilmington, Del.	Mite, cat, cockroach	6.2-16.3†; $p < 0.001^*$
Call et al. 1992	Atlanta, Ga.	Mite, cockroach	9.5†; $p < 0.001^*$
Duff et al. 1993	Charlottesville, Va.	Mite	4.5*
Sporik et al. 1993	Poole, UK	Mite and cat	$p < 0.001^*$; $p < 0.001†$
Pollart et al. 1989	Charlottesville, Va.	Mite, cat, cockroach	$p < 0.001^*$
Nelson et al. 1996	Tampa, Fla.	Mite	$p < 0.001†$
<i>Prospective studies</i>			
Ashad et al. 1992 a, Hjide et al. 1994 }	Isle of Wight, UK	Mite, cat	6.1‡; $p < 0.01‡$; 16.1‡
Sporik et al. 1990	Poole, UK	Mite	19.7*; 4.8†
Sears et al. 1989	Dunedin, New Zealand	Mite, cat, dog	6.7, 4.2, 3.7*
Arshad et al. 1992 b	Isle of Wight, UK	Mite, cat	3.2*; $p = 0.002^*$

*Sensitization (IgE antibodies) alone.

†Sensitization and exposure.

‡Exposure as a risk factor for sensitization.

Virginia.²⁸ We find it striking that in each of these analyses, sensitization to pollen allergens does not appear to be an independent risk factor for asthma.^{27, 28, 168, 169} The relevance of other indoor allergens is not consistent and depends both on the climate and on socioeconomic features of the community. Sensitization to cat allergens shows up as an independent variable secondary to dust mite in several analyses.^{16, 27, 28} In Scandinavia and the mountain states of the United States, sensitization to domestic animal allergens may be the strongest association with asthma.^{11, 14, 15, 87, 172} Sensitization to cockroach allergens is only relevant in communities in which cockroach infestation is common, and in some of those areas sensitization to allergens derived from *Blattella germanica* may be the strongest risk factor for asthma.^{12, 17, 22, 23, 173} Indeed, in Baltimore, Md., it was found that the size of the skin test wheal to cockroach allergen is the single strongest predictor of severity of asthma.¹⁷⁴ The National Cooperative Inner-City Asthma Study reported that children with asthma living in poverty in North American cities were commonly sensitized to multiple allergens including *Alternaria*, cockroach, and dust mites. Among these children, the combination of sensitization and exposure to cock-

roach allergens was strongly associated with increased hospitalization for asthma.¹⁷ The data are striking because in the United States (though not in other countries), children of lower socioeconomic groups have higher rates of morbidity and mortality from asthma than children in higher socioeconomic groups regardless of race or ethnic background.¹⁷⁵

Allergen exposure and asthma symptoms

The relationship between exposure to indoor allergens and symptoms of asthma is complex. That there is not a close relationship is clear from the observation (seen in many studies) that approximately 50% of the allergic children and young adults who are exposed to relevant allergens in their houses do not wheeze.^{158, 176} The factors that influence the relationship between exposure and symptoms include (1) interaction between the effects of exposure to several different allergens, (2) the influence of multiple factors that can enhance the inflammatory response to allergens, (3) the lack of a simple objective test that characterizes asthma or inflammation of the lungs, and (4) the many triggers and enhancers that can influence narrowing of the airways or the perceived severity of symptoms.

In some studies, a good dose response has been seen with symptoms; however, this has generally involved comparing different climatic areas.^{164-166, 177} This problem has recently been discussed in an editorial addressing the reasons why there is not a clear dose-response relationship between the level of allergen in houses and symptom severity¹⁶² (Figure).

The prevalence of asthma has increased in Westernized countries. Obviously, this conclusion is most convincing in those studies in which standardized methods have been used to document the prevalence.¹⁷⁸⁻¹⁸⁰ What is clear is that the documented increases are in perennial rather than seasonal asthma and that in all studies in which appropriate skin testing or serum assays have been carried out, there has been a very high prevalence of sensitization among the asthmatic subjects. However, the role that increase in exposure to allergens has played is less clear. In Australia, there is evidence for an increase in mite allergens over the period when prevalence has increased.^{168, 179} However, in that country the increased prevalence of asthma symptoms appears to reflect an increase in BHR rather than an increase in sensitivity among children allergic to mites. By contrast, in the United Kingdom it seems unlikely that there was a significant increase in mites or mite allergen over the decade of 1979 to 1989.^{161, 180} The simple argument that increased exposure to indoor allergens has been the driving force behind increased prevalence of asthma also would have to explain the increase in asthma prevalence in communities in which cockroaches or domestic animals are the dominant indoor allergens.

The argument that indoor allergen exposure is causally related to asthma is most complete for house dust mites. For the other indoor allergens, there is good evidence about the association between sensitization and asthma (Table II) and some evidence about exposure levels.^{11, 15, 17, 87} For cat allergens, there is evidence that cumulative duration of exposure is a determinant of sensitization. In other studies there has been little or no relationship between the quantity of cat allergen in house dust and symptoms or sensitization.^{11, 87} A very important question appears to be whether exposure to animal allergens should be evaluated on a community basis or in individual houses. Thus in a comparison of an inner city community to suburban homes, there are clear differences in mean levels of cockroach or cat allergens that directly relate to sensitization and the associated risk of asthma.²² However, the concentration of cat (Fel d 1) or cockroach (Bla g 2) allergen in dust from individual houses within a community does not correlate well with sensitization or symptoms.^{11, 15, 28, 87} In areas where both dust mites and other allergens are present in houses, analysis of the relative risk related to each allergen is not possible.^{14, 181, 182} Apparent differences in the importance of different allergens may be a factor of exposure levels, the size of airborne particles, or the inherent potency of the allergen source. Although accurate measurement of allergens in reservoir dust is the best technique available for quantitating exposure in

Sensitizers, Enhancers and Triggers for Asthma

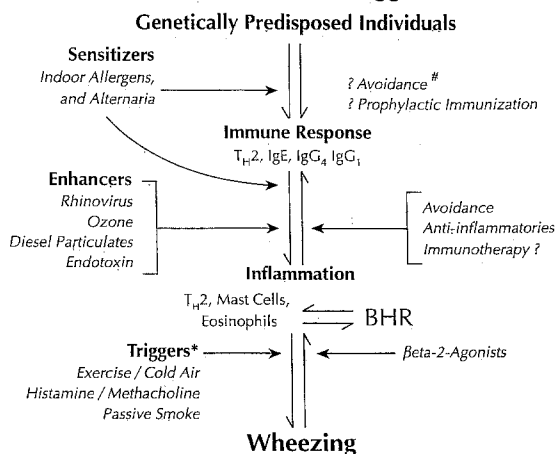


FIG. 1. Sensitizers, enhancers, and triggers for asthma. #Allergen avoidance and prophylactic immunization have been proposed as methods of preventing or modulating the immune response to allergens. *Both sensitizers and enhancers can act as triggers in a hyperreactive subject.

epidemiologic surveys, it would be simplistic to imply that the effect on the respiratory tract of 1 µg of group 1 mite allergen was equal to that of 1 µg of Fel d 1 or 1 µg of Bla g 2.

Other factors that may influence the relationship between allergen exposure, bronchial hyperreactivity, and symptoms of asthma

Although the primary concern of this report is to discuss the role of allergens in asthma, it clear that many other factors can influence the response to allergens. Experimental studies have shown that diesel particulates can have a direct effect on respiratory epithelium.¹⁸³⁻¹⁸⁵ For ozone, the effect appears to be an enhancement of the response to allergen challenge.^{186, 187} Overall, the epidemiologic evidence relating to the effects of air pollution on asthma has been modest.^{188, 189} A high prevalence of asthma has been observed in countries such as New Zealand, where outdoor air pollution is not a significant factor. Enhancing effects have been reported with endotoxin,¹⁹⁰ rhinovirus,¹⁹¹⁻¹⁹⁴ and chronic use of β₂-agonists.¹⁹⁵ The important feature is that the proinflammatory effects of these enhancers are in general dependent on concomitant allergen exposure^{186, 191, 195} (Figure).

If the increases in asthma and BHR have been greater and more consistent than can be explained simply by the increases in indoor allergen exposure, it is logical to search for other changes that could have led to a generalized increase in reactivity. Several groups have pointed out that changes in diet in Western society have been ubiquitous and could have played a role in increased immune responses. Regular fish intake has been shown to have a protective effect against BHR.^{196, 197} By contrast, a high salt diet is thought to increase the severity of asthma.^{198, 199} In many West-

ern countries there has been a progressive decline in the consumption of fresh fruit and vegetables, often with a parallel increase in sodium intake.^{200, 201} In some populations these changes could have been sufficient to make an impact on the prevalence asthma; however, most investigations consider that the dietary changes that have occurred are not sufficient to explain either the consistency (over 30 years) or the magnitude of the increases in asthma. It has also been proposed that recurrent viral or bacterial infections in early childhood could alter the balance between T_{H1} and T_{H2} responses.²⁰²⁻²⁰⁴ Bacterial infection in general suppresses the development of T_{H2} responses. This suggests that decreases in the duration and severity of bacterial infections (as the result of increased use of antibiotics) could have increased the prevalence of sensitization. The situation for viral infections is less clear because viral infections may either promote or suppress the development of T_{H2} responses.

Genetics

Genetics was not a focus of the workshop. However, there are many different genetic influences over immune responses that are highly relevant to asthma. Some of the genetic studies have suggested that the association between total IgE and asthma might be due to a hyperresponsive cytokine system causing both airway inflammation and nonspecific increases in IgE. In those studies in which both asthma and IgE antibodies are documented objectively, specific IgE antibody to indoor allergens has been shown to be the dominant risk factor whereas total IgE is either a secondary factor or not an independent risk factor for asthma. Thus it is clear that all studies on the genetics of asthma should identify and measure IgE antibodies to the environmental allergens that are relevant to asthma in that population.

Conclusions and research issues

The association between sensitization to indoor allergens and asthma has been observed with domestic animal and cockroach allergens as well as dust mite allergens. The association is both consistent, that is, in many different countries, and very strong (adjusted odds ratios from 3 to ≥ 6). The results show that sensitization to foreign proteins inside houses is a (or the) major risk factor for asthma and that the relationship between exposure to these allergens and asthma should be considered to be causal.

Many different factors can upregulate inflammation in the lungs experimentally; these include rhinoviruses, diesel particulates, and ozone. In addition, endotoxin can increase bronchial reactivity in patients allergic to mites. In each case, these "enhancers" act selectively on allergic patients rather than inducing an immune or inflammatory response.

It has also been postulated that a nonspecific effect on immune responsiveness could explain the increase in prevalence of asthma and allergic disease. The most convincing possibilities are the effects of diet and/or the

effects of widespread use of broad-spectrum antibiotics. At present, none of these adequately explains the increase in asthma that has occurred in different countries and different socioeconomic groups.

If the increase in asthma over the period 1960 to 1995 is due to an increase in exposure to allergens, it would be necessary to assume that several different indoor allergens have increased in parallel, that is, mite, cat, dog, and cockroach.

Recommendations

The interaction between allergen exposure and other factors that can influence inflammation of the lungs (including the currently identified enhancers) must be studied in relation to both prevalence and severity of asthma. This will require experimental, cross-sectional, and population-based studies. Specifically, we need data on the interactions between viral infections and allergen exposure in children and adults.

Population studies are needed to answer whether the genetic controls over total serum IgE, IgE antibody responses, and asthma act independent of the effects of exposure to indoor allergens.

Studies on the increased prevalence and severity of asthma should investigate whether the increase represents (1) increased sensitization of the community, (2) increased inflammation and/or BHR among the atopic individuals, or (3) increased symptom severity among patients who have BHR.

ALLERGEN AVOIDANCE

The control of allergen exposure is an ongoing process that requires (1) a strategy to address the known sites where the allergens occur, (2) the use of multiple short-term and long-term control methods, and, where possible, (3) measurement of allergen exposure to identify sources within the house and to monitor the effectiveness of control measures. Several studies have proposed exposure thresholds for different allergens. Sensitized individuals vary widely in their susceptibility to levels of exposure, and no absolute value that could function as a recommended target has been identified. The recommendation of a 90% reduction as a feasible and appropriate aim for control measures was adopted.^{1, 2} Intervention methods can influence the accurate assessment of allergen exposure. Allergen exposure is currently expressed as micrograms of allergen per gram of dust, or micrograms of allergen per square meter. The former is more appropriate for epidemiologic studies, whereas either or both may be used for intervention studies, depending on the control procedures used.

Clinical trials

The benefits from long-term reduction in exposure, obtained by moving patients to a low-allergen environment, have been consistent (see References 1 and 2). These benefits include reductions in both specific and total IgE^{205, 206}; improvements in airway hyperreactivity, peak flow values, and FEV₁^{207, 208}; and a reduction in the

eosinophil phase of airway inflammation.²⁰⁹ The respiratory parameters were improved in some but not all cases.²⁰⁵ In a group of subjects with severe asthma, a reduction in steroid use in those predominantly sensitized to indoor allergens but not in those with persistent fungal infections was observed.²¹⁰ Prolonged allergen avoidance does not diminish allergen sensitivity, and subsequent high allergen exposure will generally result in recurrence of symptoms.²⁰⁷ Recent trials in houses have demonstrated clinical benefits in asthma^{211, 212} and atopic dermatitis.¹⁵² These studies used a combination of methods including bedding encasings, acaricides, and various hygiene methods, and it is difficult to separate out the contribution of individual methods to reducing symptoms. A prospective trial of at-risk infants, combining allergen avoidance with dietary manipulation during the first year, showed a reduction in the incidence of sensitization and a trend toward less asthma.^{213, 214}

Hygiene control of allergens

Beds. The effectiveness of controlling mite allergens in beds by using encasings is now well established.^{212, 215-218} This is in keeping with the fact that the primary evidence about the contribution of mite allergens to airway disease relates to bedding. Encasings for bedding that are permeable to air and water vapor but not to mites or allergens are widely available and are more comfortable than those that are completely impermeable. Covers should be chosen to be robust, easily fitted, and easily cleaned. Allergens continue to accumulate on top of and inside the encasings.²¹⁹ If encasings are only fitted to the mattress and pillows, it is important that all bedding be regularly washed to remove accumulated allergens.²²⁰

Floor coverings, furnishings, and other sites. Other important sites where mite allergens accumulate include floor coverings, soft furnishings, mattress box springs, toys, and clothing.²²¹⁻²²³ Where allergen levels in carpets are high, it is difficult to obtain a large reduction (> 80%) or to maintain reductions for more than 3 months with simple and economic treatments. These generalizations about carpet treatment are true for heated steam, wet or dry vacuum cleaning, acaricide treatment, or tannic acid treatment. If other options such as carpet removal are not feasible, each of the above methods may have some effect and may be useful if used in combination. However, it was generally agreed that in humid climates, removal of carpets is the best option.

Laundry. As previously described, washing in hot water (>55° C) is preferred because it both removes allergen and kills mites. Washing in cooler water does not kill all mites but is an effective way to remove allergens, particularly if used more frequently. A soluble form of benzyl benzoate (final concentration 0.03%) can be used in warm and cool water to kill mites, as can some essential oils (at 0.2% final concentration).^{224, 225} Dry cleaning kills all mites and removes dust, but it does not destroy all allergens.^{226, 227} Tumble drying can kill all mites if a

temperature greater than 55° C is maintained for 10 minutes.²²⁸ It is important when choosing bedding, clothing, furnishings, and so forth that the materials will withstand regular washing or cleaning.

Vacuum cleaners. Regular vacuum cleaning is important to minimize the total allergen burden, but its effect is unlikely to be sufficient to control exposure from carpets and furnishings.²²⁹ Further data have been reported on the generation of airborne allergen particles by both wet and dry vacuum cleaners, and large differences in performance between models have been demonstrated.²³⁰⁻²³² Recommendations are for the use of vacuum cleaners where the bag has two layers, or high-efficiency particulate air (HEPA) or electrostatic filtration systems on the exhaust air. Ducted cleaning systems that exhaust out of the house may offer similar advantages. Steam cleaning of carpets can kill mites and remove allergen, but steam generally does not penetrate to the bottom layers of the carpet, and it is difficult to remove residual water so that steam is usually only effective for a short period of time.^{233, 234}

Acaricides and denaturants. While some controlled studies with acaricides have shown reductions in allergen levels,^{235, 236} others have shown insufficient reduction²³⁷⁻²⁴⁰ to justify their use. With acaricides, not all mites are killed, particularly in the layer underneath the carpet, and allergen levels in carpets and furnishings only fall slowly. Conversely, with tannic acid, substantial initial reductions in allergens are obtained, but the benefits are temporary.²⁴⁰⁻²⁴² With both acaricides and agents designed to denature allergens, repeated application is required to maintain the effects. Cases of skin sensitization have been reported among research personnel applying acaricides repeatedly, but no serious health risks of these products have been identified among occupants of treated houses. While opinion of the participants was divided, the majority believed that these agents only have a limited role in controlling mite allergens.

Freezing and solar exposure. Freezing in a domestic freezer for 24 hours provides an efficient method of killing mites²⁴³ in small articles such as soft toys and special clothing. Freezing carpets with liquid nitrogen is also effective but not in widespread use. Placing carpets in the direct sun for several hours can create conditions of high temperature and low humidity which are rapidly fatal for mites and eggs.²⁴⁴ Solar exposure has no short-term effect on mites in mattresses, which are too thick, or in vertically hung blankets, as the latter equilibrate with ambient conditions. All these measures only kill mites, and other measures such as washing or beating are required to remove the allergens.

Ionizers and air filtration devices. Despite widespread use and promotion, there is currently too little supportive data or rationale to justify recommending the use of ionizing or air filtration devices to control mite allergens.²⁴⁵⁻²⁴⁸ Such devices do not affect the large reservoirs of mite allergen that function as local sources of exposure, and primary control efforts should be directed at controlling these.

Control of cat and dog allergens

The primary treatment for patients with allergic disease who are sensitive to cats and dogs should be the removal of the pet. However, in many and perhaps a majority of cases, the family will not agree to this. Methods for removing animal allergen from a house or controlling allergen in the presence of an animal reflect the distribution of these allergens in the house. Animal dander allergens are generally highest in living rooms or on furniture and lower in bedrooms.^{22, 87, 154, 249} Cat and dog allergens often reach higher concentration in dust than mite allergens.^{22, 87, 250} Compared with mite allergen, animal aeroallergens are associated with smaller particles that remain airborne longer or even continuously; thus there is a rationale for using air filtration.

The extent to which exposure to cat allergens can be effectively controlled by methods such as removing reservoirs (such as carpets and sofas), cat washing, HEPA filtration, topical sprays, or even castration of the cat is unclear. Some studies have suggested that these procedures are effective, but others suggest that the effects are limited.²⁵¹⁻²⁵⁵ Washing cats can remove as much as 10 mg Fel d 1 from the cat, and allergen becoming airborne off the cat is decreased after washing but will generally reaccumulate within 1 week. Regular use of the combination of washing, removing reservoirs, and air filtration has shown clinical efficacy in two studies.^{253, 256} This requires a high level of dedication and may be an effective method for persuading the family to relocate the animal.²⁵⁶ Minimizing total exposure to cat and dog allergens also may require addressing reservoirs of such allergens in schools,²⁵⁷⁻²⁶⁰ occasionally in other public buildings,²⁶¹ and in houses with no record of pet occupancy.²⁶² Pet allergens are presumably transferred to these sites on clothing.

Future trials

There are two situations in which allergen avoidance should be considered. These are (1) in infants at high risk of developing allergic disease to prevent or delay sensitization (primary prevention) and (2) in patients with established disease in whom sensitization has already occurred (secondary prevention). There is an urgent need to develop adequately powered, randomized, controlled studies to investigate the potential benefits of low-allergen domestic environments in patients with allergic diseases. Such studies must address compliance and cost-effectiveness, must be of adequate length (e.g., 12 months), and must be tailored for different socioeconomic groups and age groups. Avoidance of primary sensitization in susceptible infants is currently being addressed by several large trials.

Regulation

The allergen avoidance market continues to be supplied with an increasing number of products claiming to control mites and various allergens. Currently, there is little or no regulation governing such claims. All products should have demonstrations of their efficacy in

relation to mites and allergens in the home environment as well as safety published before they are released on the market. They should provide clear instructions for their use and list likely or known potential hazards (e.g., staining, smell, allergen dispersal). Appropriate standards for products marketed for allergen avoidance should be developed by industry or consumer agencies.

Housing design as an ecologic control of mites and allergens

An alternative or addition to hygiene approaches for minimizing exposure (as above) is to create general ecologic conditions that counter the biological factors that drive mite populations and allergen production at any site.^{1, 2} Numerous recent studies confirm the association between indoor humidity and mite allergen levels.^{259, 261, 262} Further indirect associations with humidity include altitude of building floors,^{261, 262} ventilation rates,^{223, 262} observed dampness,²⁶¹ use of concrete floors,²⁶³ and high occupant densities.²²³ Calculations for optimizing air exchange rates for houses to provide a continuous absolute indoor humidity of 7.0 grams or less of water per kilogram of dry air have been provided (see Korsgaard, UCB Document). This requires a reasonable housing standard without water leakage or excessive ground humidity. In cooler climatic regions, an additional heat recovery system would be required for energy efficiency; in humid regions (outdoor water content > 5.5 g/kg), an additional dehumidification system would be required. However, in hot climates, buildings are generally not airtight, and attempts to apply dehumidification or air conditioning have not always been useful in controlling mites.^{264, 265}

The concentrations ($\mu\text{g/g}$) of mite allergen within a community show wide variations between houses that are not always related to average levels of indoor humidity.^{166, 223, 266-268} This suggests that additional ecologic factors (nutrition, habitat, etc.) may be important and that low allergen exposure may be possible in any climate if the appropriate principles are identified and exploited.

SUMMARY

The major allergens from dust mites, cat, dog, and German cockroach have been identified, cloned, and sequenced. Many of these allergens can be expressed as recombinant proteins, which, with some exceptions, have strong reactivity with IgE antibodies. Detailed analyses of IgE binding and T-cell responses have been carried out. Studies to define the tertiary structure of some of these proteins are in progress.

Sensitive and precise immunoassays to measure indoor allergens have been developed. Monitoring exposure is primarily dependent on measuring allergen levels in dust samples obtained from reservoirs within the house, that is, carpets, bedding, sofas, or floors and expressing the results as micrograms per gram of dust. For cat and dog allergens, measurements of airborne levels also can be used to monitor exposure. In some circumstances, particularly during avoidance studies,

measurements of total allergen recovered from an area or site also may be relevant.

There is a dose-response relationship between exposure to dust mite allergens in the home and sensitization to mites. In areas where the mean level of dust mite group 1 allergens in houses is 2 $\mu\text{g/g}$ or more, sensitization to mites has consistently been found to be associated with asthma. For cat and cockroach allergens, evidence about the exposure necessary to induce sensitization has come from comparing communities without exposure or sensitization with those that have significant exposure and sensitization.

The increase in asthma prevalence has occurred mainly among patients experiencing perennial rather than seasonal symptoms and has been strongly associated with sensitization to indoor (rather than outdoor) allergens. In humid areas of the world, sensitization to mite allergens is the most significant risk factor for asthma when adjusted odds ratios are compared. However, in many less humid areas of the world, increases in asthma or a very high asthma prevalence has been documented in which the relevant indoor allergens are derived from domestic pets or cockroaches rather than dust mites.

Symptom severity among allergic individuals is not closely correlated with the concentration of allergen in house dust because several other factors that affect the lung can act to enhance inflammatory responses to inhaled allergens. Experimental rhinovirus infection, continuous use of β_2 -agonists, endotoxin, and ozone each have been shown to enhance the late response to bronchial challenge with allergens. The symptoms of asthma will thus depend on exposure to sensitizers (e.g., indoor allergens), enhancers, and traditional triggers (e.g., cold air, histamine, exercise, or emotions, which can produce acute effects without a prolonged inflammatory effect).

Allergen-specific treatment for asthma consists of allergen avoidance and immunotherapy. (1) Avoidance measures can be effective if they are pursued aggressively, but implementation of these procedures can be difficult, and further research is required to improve avoidance strategies. In particular, there are currently only partial solutions to reducing mite allergens in carpets or sofas that are kept in humid conditions. The control of animal allergens, short of removing them from the house if not the community, also requires further research. (2) The use of traditional immunotherapy in moderate to severe perennial asthma remains controversial; however, cloning of the major indoor allergens has led to promising research on peptides that react selectively with T cells and allergens mutagenized to reduce reactivity with IgE antibodies. The development of adjuvants that are capable of modifying responses of allergen-reactive T cells *in vivo* is eagerly awaited.

Although there is clear evidence that exposure to indoor allergens is the primary cause of T_{H2} and IgE responses in patients with asthma, documented increases in exposure to the different indoor allergens are not adequate to explain the increase in sensitization and symptoms. It remains an important goal to identify the factors that are responsible for the increased incidence of immunologic sensitization

Table III.

Co-Chairmen:

Thomas A. E. Platts-Mills
Daniel Vervloet
Wayne R. Thomas
Robert C. Aalberse
Martin D. Chapman

Participants:

Carlos Baena-Cagnani, Cordoba, Argentina
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Atillio Boner, Verona, Italy
K. Bruinzeel-Koomen, Utrecht, The Netherlands
Luis G. Caraballo, Cartagena, Colombia
Fleming Carswell, Bristol, U.K.
Dennis Charpin, Marseille, France
Kaw Yan Chua, Taipei, Taiwan
Matthew Colloff, Canberra, Australia
Julian Crane, Wellington South, New Zealand
Oliver Cromwell, Hamburg, Germany
Adnan Custovic, Manchester, U.K.
Sten Dreborg, Oslo, Norway
Lena Elfman, Uppsala, Sweden
Peyton A. Eggleston, Baltimore, Md.
Enrique Fernandez-Caldas, Madrid, Spain
Diane R. Gold, Boston, Mass.
Marianne van Hage-Hamsten, Stockholm, Sweden
Peter W. Heymann, Charlottesville, Va.
Chein-Soo Hong, Seoul, Korea
Martien Kapsenburg, Amsterdam, The Netherlands
Frans Kniest, Reinbek bei Hamburg, Germany
Jens Korsgaard, Aalborg, Denmark
Henning Lowenstein, Horsholm, Denmark
Christina Luczynska, London, U.K.
Terry Merrett, Oxford, U.K.
Jeffrey Miller, Ridgefield, Conn.
E. Bruce Mitchell, Dublin, Ireland
Frederico Montaleagre, Ponce, PR
Geert Mudde, Vienna, Austria
A. K. M. Munir, Linkoping, Sweden
Erica von Mutius, Munchen, Germany
Charles Naspietz, Sao Paulo-SP, Brazil
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J. E. M. H. van Bronswijk, Eindhoven, The Netherlands
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Ulrich Wahn, Berlin, Germany
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and the increased severity of clinical symptoms in sensitized patients.

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