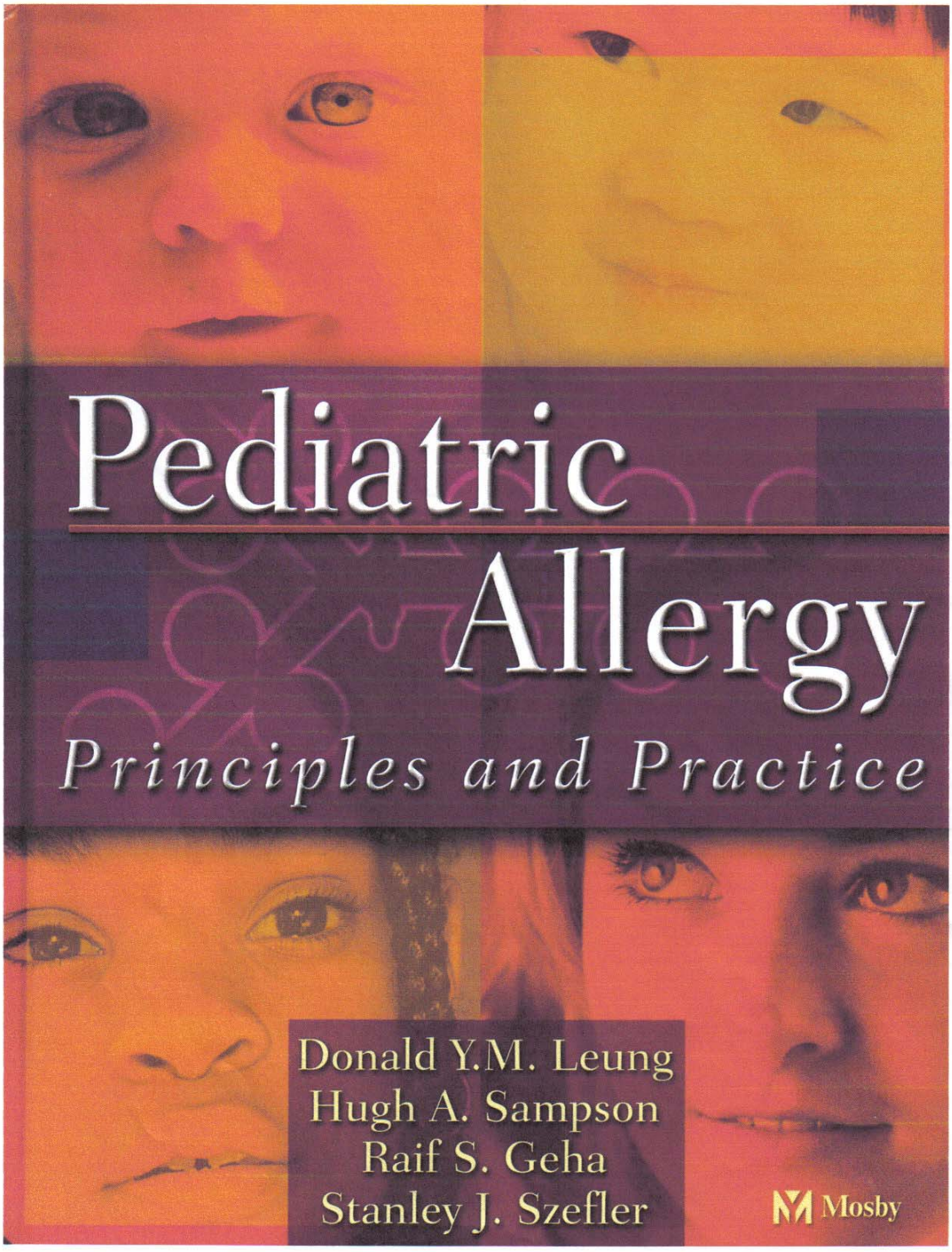
Pediatric
Allergy
Principles and Practice
Donald Y.M. Leung
Hugh A. Sampson
Raif S. Geha
Stanley J. Szefler
Mosby

CHAPTER 25

Indoor Allergens

MARTIN D. CHAPMAN

House dust allergens have been associated with asthma since the 1920s, when Kern[1] and Cooke[2] independently reported a high prevalence of immediate skin tests to house dust extracts among patients with asthma. At the same time, Van Leeuven[3] showed that asthma patients who were admitted to a modified hospital room free of "climate allergens" (which he believed to be bacteria and molds) showed clinical improvement: some of the first experiments using allergen avoidance for asthma management. In the mid twentieth century, allergists sought to explain how such a heterogenous material as house dust could contain a potent allergen that appeared to be ubiquitous in dust extracts. The prevailing theory was that a chemical reaction occurred in dust resulting in the synthesis of the "house dust allergen." Researchers extracted house dust with phenol and other solvents to identify allergenically active compounds.[4] The puzzle was finally resolved in the 1960s, when Voorhorst and Spieksma[5] showed that the origin of house dust allergen was biologic rather than chemical. The allergenic potency of Dutch house dust extracts correlated with the numbers of house dust mites in the samples and extracts of pure mite cultures (Acari, Pyroglyphidae: *Dermatophagoides pteronyssinus* and *D. farinae*) gave positive skin tests at dilutions of 10^{-6} or greater.[5] Voorhorst and Spieksma[5] also showed a correlation of asthma symptoms with seasonal variation in mite numbers, providing the first evidence for exposure thresholds: exposure to 100 mites per gram of dust was associated with sensitization, and 500 mites per gram was associated with symptom exacerbation.

The prevalence of asthma has increased during the past 40 years, and current data suggest that approximately 10% of U.S. children have asthma. Sensitization and exposure to indoor allergens, principally dust mites, animal danders, cockroach (CR), and fungi, are among the most important risk factors for asthma.[6-9] The two principal mite species, *D. pteronyssinus* and *D. farinae,* account for more than 90% of the mite fauna in U.S. house dust samples. Other allergenic mites include *Euroglyphus maynei* and *Blomia tropicalis* (found in subtropical regions such as Florida, southern California, Texas, and Puerto Rico). Storage mites, such as *Lepidoglyphus destructor, Tyrophagus putrescentiae,* and *Acarus siro,* may cause occupational asthma among farmers, farm workers and their families, and grain handlers. The relationship among exposure, allergen sensitization, and asthma has been most thoroughly explored for dust mite allergens. Epidemiologic studies in many parts of the world have established that exposure to 2 μg /g mite group 1 allergen in the dust results in allergic sensitization and that there is a dose-response relationship between exposure and the numbers of atopic individuals who become sensitized.[6-9] There is further evidence that asthma severity is related to mite allergen exposure.[10,11]

Childhood asthma is also strongly associated with sensitization to animal allergens, CR, and, to a lesser extent, mold allergens. Cat allergen (Fel d 1) has a ubiquitous distribution in the environment and can be found at clinically significant levels in houses that do not contain cats (similarly for dog allergen).[12-14] Rodent urinary proteins have long been associated with occupational asthma among laboratory animal handlers; recently a high prevalence of sensitization and exposure to mouse allergen was reported among inner-city children with asthma.[15] These children are also at the greatest risk of developing CR allergy. CR infestation of housing results in the accumulation of potent allergens that are associated with increased asthma mortality and morbidity among U.S. children, particularly African-American and Hispanic children, living in inner cities.[16,17] CR allergens appear to be particularly potent. Atopic individuals develop IgE responses after exposure to tenfold to a hundredfold lower levels of CR allergens than to dust mite or cat allergens.[18] Asthma is the most common disease associated with CR and occurs in CR-infested housing in inner cities, as well as in suburbs and rural areas.

Investigation of the role of indoor allergens in asthma has involved the identification of the most important allergens and the development of techniques to accurately monitor allergen exposure. This chapter reviews the structure and biologic function of indoor allergens and current allergen detection systems that are used to assess exposure in the laboratory and, increasingly, by patients themselves. The interpretation of exposure results and clinical significance of allergen exposure assessments is also discussed.

ALLERGEN STRUCTURE AND FUNCTION

Allergens are proteins or glycoproteins of 10 to 50 kDa that are readily soluble and able to penetrate the nasal and respiratory mucosae. Molecular cloning has determined the primary amino acid sequences of more than 500 allergens (see http://www.allergen.org), and 20 to 25 three-dimensional structures of indoor allergens have been resolved.[19] Structural analyses have not revealed any common features or motifs that are associated with the induction of IgE responses

BOX 25-1 KEY CONCEPTS

Indoor Allergens: Structure and Function

- **Allergens are soluble proteins or glycoproteins of molecular weights of 10 to 50 kDa.**
- **Most major allergens have been cloned, sequenced, and expressed.**
- **More than 500 allergen sequences are deposited in protein databases (GenBank, PDB), and about 20 tertiary structures have been resolved by x-ray crystallography.**
- **Allergens have diverse biologic functions and may be enzymes, enzyme inhibitors, lipocalins, or regulatory or structural proteins.**
- **Allergens promote T cells to differentiate along the Th2 pathway to produce IL-4 and IL-13 and to initiate isotype switching to IgE.**

(reviewed in Chapman et al[19] and Aalberse[20]). Allergens belong to several families of proteins and have diverse biologic functions: they may be enzymes, enzyme inhibitors, ligand-binding proteins, or structural or regulatory proteins (Box 25-1). A systematic allergen nomenclature has been developed by the International Union of Immunological Societies' (IUIS) Allergen Nomenclature Subcommittee: the first three letters of the source genus followed by a single letter for the species and a number denoting the chronologic order of allergen identification. Thus the abbreviated nomenclature for *Dermatophagoides pteronyssinus* allergen 1 is Der p 1. To be included in the IUIS nomenclature, the allergen must have been purified to homogeneity and/or cloned, and the prevalence of IgE antibody (ab) must have been established in an appropriate allergic population by skin testing or *in vitro* IgE ab assays.

Most dust mite allergens are digestive enzymes excreted with the feces, such as Der p 1 (cysteine protease), Der p 3 (serine protease), and Der p 6 (chymotrypsin). With the exception of cat allergen Fel d 1, most animal allergens are ligand-binding proteins (lipocalins) or albumins. Lipocalins are 20- to 25-kDa proteins with a conserved, eight-stranded, antiparallel β-barrel structure; they serve to bind and transport small hydrophobic chemicals[21] (Figure 25-1). As a group, lipocalins have a variety of ligand-binding functions, but the rat and mouse urinary allergens are pheromone- or odorant-binding proteins, and it is likely that the other lipocalin animal allergens have similar functions. The CR allergen Bla g 4 is also a lipocalin. Other important CR allergens include Bla g 2, an inactive aspartic proteinase, Bla g 5 (glutathione transferase family), and Per a 7 (tropomyosin).[17] Fungal allergens have been cloned from *Alternaria, Aspergillus, Cladosporium, Penicillium,* and *Trichophyton* spp., and several of these allergens are proteolytic enzymes, heat shock proteins, or ribonucleases.[22] There is conflicting evidence as to whether biologic function influences allergenicity. Some researchers have proposed that the enzymatic activity of mite allergens promotes IgE synthesis and local inflammatory responses via cleavage of CD23 and CD25 receptors on B cells and by causing the release of pro-inflammatory cytokines (interleukin [IL]-8, IL-6, monocyte chemotactic protein-1 [MCP-1], and granulocyte-monocyte colony-stimulating factor [GM-CSF]) from bronchial epithelial cells. Mite protease allergens cause detachment of bronchial epithelial cells *in vitro* and disrupt intercellular tight junctions.[23,24] On the other hand, several potent allergens, including Der p 2, Fel d 1, Bla g 1, and Bla g 2, have no enzymatic activity, and some animal allergens (Can f 1 and Fel d 3) are cysteine protease inhibitors.[21]

Recombinant allergens have been produced in high-level expression systems in bacteria (*Escherichia coli*), yeast (*Pichia pastoris*), and baculovirus. In general, the allergenic activity of the recombinant allergens, assessed by skin testing and *in vitro* IgE antibody assays, is comparable to that of the natural allergens. The advantages of recombinant allergens are that they can be precisely manipulated, targeted, and engineered and they can be formulated at defined concentrations and potency.[19] Cocktails of two to four major allergens can be used

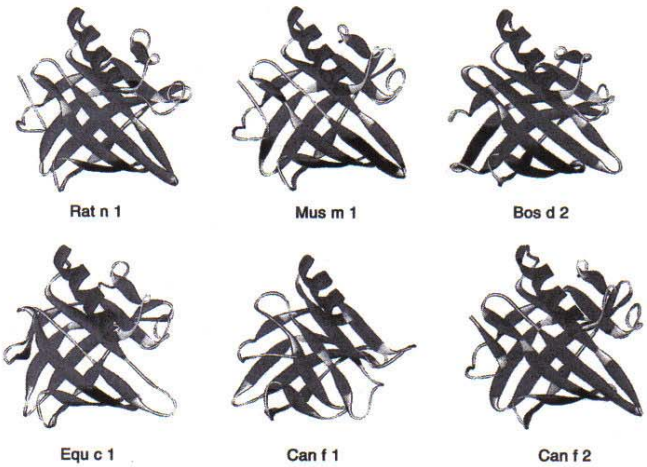

FIGURE 25-1 Molecular structures of the lipocalin allergens. Each lipocalin comprises eight antiparallel β sheets and an N-terminal α helix. (From Pomés A, Smith AM, Grégoire C, et al: *Allergy Clin Immunol Int* 13:162-169, 2001.)

for diagnostic purposes with the same specificity and sensitivity as natural allergen extracts. Recombinant allergens are being used to develop new generations of diagnostic tests, including microarrays and lateral flow devices, that could ultimately replace skin tests for allergy diagnosis.[25] They are also being used to develop new therapeutic strategies and vaccines for the treatment of allergic disease.

EVALUATION OF ALLERGEN EXPOSURE

Allergen Detection Systems

Biologists and pest management companies have counted dust mites in dust samples and trapped CR to assess infestation and allergen exposure. Although these methods are useful in studying population dynamics, seasonal variation, and the effect of physical and chemical methods for reducing mite and CR populations, they are time consuming and unsuitable for routine measurements of allergen exposure. Moreover, allergen levels may remain high when mite or CR levels have been reduced, and simply enumerating mite/CR may not be a reliable indicator of allergen exposure. Since the mid-1980s, quantitative assessments of allergen exposure have been made by measuring major allergens in reservoir dust samples (bed, carpet, soft furnishings) using monoclonal antibody (mAb)–based enzyme-linked immunosorbent assays (ELISAs). The advantages of these assays are high sensitivity (~1 ng/ml), high throughput (hundreds of samples per day), accurate quantification, and defined specificity. The ELISAs for indoor allergens use either pairs of mAbs directed against nonoverlapping epitopes on the allergen molecule or capture mAb and polyclonal rabbit antibody for detection. Table 25-1 lists the antibody combinations and allergen standards for 17 ELISA systems for indoor allergens.[26] Critical elements in the development of ELISA tests are the use of high-affinity mAb of defined specificity and standards with known allergen content. A few national and international standards have been produced for the calibration of Der p 1, Can f 1, and Fel d 1 assays, and other standards are commercially available (Table 25-1). In most cases, these standards are allergen extracts that were calibrated to contain a known amount of allergen, but they are not purified allergens. Standards are important because they enable allergen measurements made by different laboratories to be directly compared. The World Health Organization (WHO)/IUIS Allergen Standardization Committee has initiated a multicenter project, involving researchers from academia, industry, and regulatory agencies, to develop international standards for purified allergens. This program is being coordinated through the European Union Certified Reference Materials for Allergenic Products (CREATE) project. The aim is to develop recombinant allergen standards for mite group 1 and group 2 allergens, as well as birch, rye grass, and olive pollen allergens. The purified allergens will be assessed for protein content and allergenic activity and will serve as primary standards for immunoassays.

Indoor allergen measurement by ELISA is the gold standard for exposure assessment; a growing number of academic and commercial reference laboratories across the United States

TABLE 25-1 Immunoassays for Indoor Allergens*

Allergen	Capture Monoclonal Antibody	Second Antibody	Allergen Standards
Mite			
Der p 1	5H8	4C1^{B}	NIBSC 82/518
	10B9	5H8^{B}	NIBSC 82/518
	PIA03	P1A01^{B}	92-Dp
Der f 1	6A8	4C1^{B}	UVA 93/02
	F1B01	F1A05	92-Df
Group 2	1D8	7A1^{B}	UVA 97/01
Group 7	WH9	HD19^{B}	NA
Blo t 5	4G9	4D9^{B}	rBlo t 5
Lep d 2	Le5B5	RaαLep d 2	rLep d 2
Mammal			
Cat Fel d 1	6F9	3E4^{B}	CBER Cat E10
Dog Can f 1	6E9	RaαCan f 1	NIBSC 84/685
Rat Rat n 1	RUP-6	RUP-1^{B}	Affinity-purified Rat n 1
Mouse Mus m 1	RaαMus m 1	RaαMus m 1	JHU
Cow Bos d 2	mAb^{3}	mAb^{1B}	nBos d 2
Horse Equ c 4	103	14G4^{B}	NA
Cockroach			
Bla g 1	10A6	RaαBla g 1	UVA 97/02
Bla g 2	8F4	RaαBla g 2	UVA 97/02
Per a 3	A-2	E-4^{AP}	Purified Per a 3
Fungi			
Asp f 1	4A6	RaαAsp f 1	Affinity-purified Asp f 1
Alt a 1	121G	121G^{B}	rAlt a 1
	Mab-1	Mab-2	Affinity-purified nAlt a 1

Modified from Chapman MD, Tsay A, Vailes LD: *Allergy* 56:604-610, 2001.

NA, Not available.

*Two-site enzyme-linked immunosorbent assay (ELISA) using capture monoclonal antibodies to coat ELISA plates and biotinylated (^{B}) of alkaline phosphatase (^{AP}) monoclonal antibody for detection of rabbit polyclonal antibody.

offer ELISA testing services. Simple qualitative or semiquantitative tests that can be used in allergy clinics or physicians' offices or by consumers have recently been developed. The aim of these "point of care" tests is to provide patients with tests that can be used to monitor allergen levels in their homes and to reinforce education about the role of allergens in causing asthma. The first such test was Acarex (Werner and Mertz, Mainz, Germany), a dipstick that measures guanine in house dust (a surrogate for dust mites), followed by DUSTSCREEN (CMG-HESKA, Freibourg, Switzerland), an mAb-based test that measures multiple allergens on a nitrocellulose strip and is designed for use in allergy clinics.[26] Recently, lateral flow technology has been used to develop rapid tests that can measure specific allergens in 10 minutes (INDOOR Biotechnologies, Charlottesville, VA). These tests are analogous to pregnancy or human immunodeficiency virus tests and are designed for use by patients and other consumers. The mite allergen test uses the same mAb as the mite group 2 ELISA and can detect both *D. pteronyssinus* and *D. farinae*. The test includes a simple dust collection and extraction device (MITEST dust collector) that allows dust to be collected and extracted within 2 minutes[27] (Figure 25-2). The rapid test has indicator lines that provide patients with estimates of high, medium, and low allergen levels; these lines have been shown to correlate with group 2 levels determined by ELISA.[27] A "wipe test" (Bio-Check [DRAGER, Lubeck, Germany]) that uses similar lateral flow technology has also been produced in Europe.[28] In principle, lateral flow tests can be applied to other allergens, and prototypes for Fel d 1 and peanut allergens have been developed.

Allergen Sampling in Dust and Air

Allergens are typically measured on dust samples that are collected by vacuuming an area of 1 m^2 for 2 minutes and extracting 100 mg of fine dust in 2 ml of buffer.[6] Samples are usually collected from three or four sites in the home, including mattresses, bedding, bedroom or living room carpet, soft furnishings, or kitchen floors. The results are expressed as nanograms or micrograms of allergen per gram of dust. Measurements of group 1 allergens in bedding provide the best index of mite exposure and show a good correlation between results expressed as micrograms of allergen per gram of dust or per unit area ($\mu g/m^2$).[29] Cat and dog allergens are widely distributed throughout the house and accumulate at clinically significant levels in houses that do not contain pets. Not surprisingly, the highest concentrations of CR allergens are usually found in kitchens, although in heavily infested homes allergen accumulates on flooring and in bedding.

Measurement of allergen levels in dust provides a valid index of exposure but cannot be used to monitor personal exposure. The aerodynamic properties of mite, cat, dog, and CR allergens have been studied using particle-sizing devices such as the Cascade impactor and Andersen sampler.[13,30-32] Mite and CR allergens occur on large particles of 10 to 40 μm in diameter and cannot be detected in rooms under undisturbed conditions. After a disturbance, such as using a vacuum cleaner without a filter, these particles remain airborne for about 20 to 40 minutes. In contrast, cat and dog allergens can be easily detected in air samples under undisturbed conditions and persist in the air for several hours. Animal dander particles (skin flakes) are less dense than mite feces, and approximately 25% of animal allergen occurs on smaller particles, 5 μm in diameter, that remain airborne. A novel personal sampling device (HALOGEN [Inhalix, Sydney, Australia]) has recently been developed that enables inhaled allergen particles to be visualized on antibody-coated slides. Silicone air samplers are worn in the nostrils for 4 to 8 hours during normal domestic activity, and inhaled particles are deposited onto the antibody-coated slides and detected by immunochemical staining. A "halo" forms on the slide around the allergen-

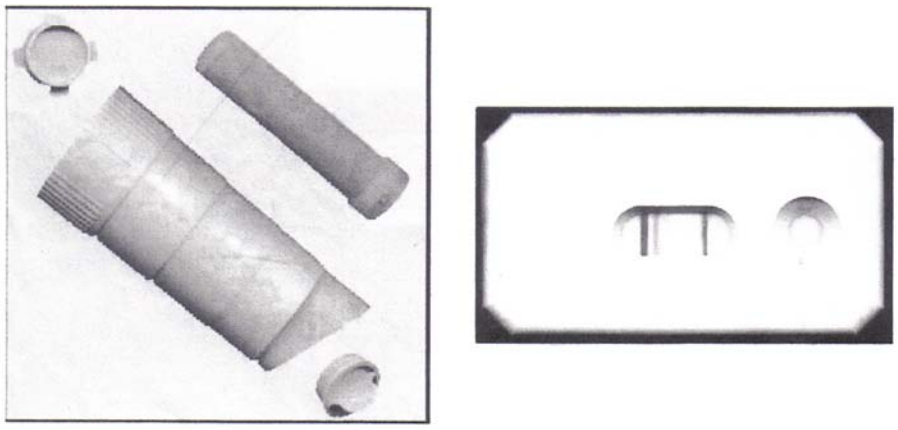

FIGURE 25-2 Rapid test for dust mite group 2 allergens. Dust samples are collected using the MITEST dust collection and extraction device *(left)*. The nylon filter is inserted into the collector and attached to the wand of a vacuum cleaner. Dust is sampled for 2 minutes and extracted in the tube. The extract is then applied to the well of the rapid test cassette *(right)*. The test shows a strong red line, and the intensity of the line can be compared with the high, medium, and low indicator lines to the left of the test line. (From Tsay A, Williams L, Mitchell EB, et al: *Clin Exp Allergy* 32:1596-1601, 2002.)

bearing particles that can be counted or the allergen can be measured using sensitive amplified ELISA systems. The HALOGEN assay has been used to monitor personal exposure to mite, cat, pollen, and fungus allergens (reviewed in O'Meara and Tovey[33]). Another new device for monitoring airborne allergen in homes is the Ionic Breeze Quadra (IBQ [Sharper Image, San Francisco, CA]), a commercial air filtration product. The IBQ is an electrostatic device that has three stainless steel blades that collect particles. The allergen can be measured by wiping the blades with filter paper and assaying the eluted allergen by ELISA. The IBQ has been shown to collect 0.5 to 8 μg Fel d 1 or Can f 1 over 24 hours and to detect mite allergens after disturbance.[34] The advantage of using the IBQ for air sampling is that it is silent and unobtrusive and can be operated in a room for several days without affecting the household or workplace.

Exposure Thresholds

Epidemiologic studies carried out in the United States, Europe, Australasia, and Japan have shown that sensitization to indoor allergens is the most significant risk factor for childhood asthma. Evidence from population surveys, case-control studies, prospective studies, and studies carried out at high altitudes or in hospital emergency departments were reviewed at the Third International Workshop in 1997.[6] The workshop confirmed that exposure to 2 μg/g mite group 1 allergens was a risk for sensitization to dust mites and that there was a dose-response relationship between the level of allergen exposure and sensitization. Adjusted odds ratios (ORs) for sensitization and exposure to 2 μg/g mite group 1 range from 3 to 6, and in many parts of the world sensitization to mites is the strongest independent risk factor for asthma. Recent studies have confirmed and extended these findings. A survey of 1054 middle school children in Virginia showed that dust mite sensitization was independently associated with asthma (OR 6.6, $P < 0.0001$) and that dust from 81% of homes contained more than 2 μg/g mite group 1 allergen.[35] A prospective study of 939 German schoolchildren followed up to age 7 showed a sevenfold difference in sensitization to mite between children exposed to less than 0.03 μg/g mite group 1 (first quartile) with those exposed to 1 to 240 μg (fourth quartile).[36]

Although the exposure data have been most thoroughly investigated for mite allergens, similar relationships between exposure and sensitization have been observed for other indoor allergens (Table 25-2). Most houses that contain cats or dogs have Fel d 1 or Can f 1 levels of greater than 10 μg/g, whereas homes that do not contain these pets may contain 1 to 10 μg/g animal allergen.[12-14] What distinguishes animal allergen exposure from other indoor allergens is the wide range of exposure levels (from < 0.5 to > 3000 μg/g) and the ubiquitous allergen distribution. Cat and dog allergens occur in schools, offices, workplaces, and public buildings, where they are passively transported by their owners, and they can cause both sensitization and symptoms in these environments.[37,38] An atopic child who lives at home without a cat can become sensitized by visiting homes or attending schools where cat allergen is present. A recent Swedish study showed a ninefold increased risk of asthma exacerbations at school among 6- to 12-year-old children who attended classes with other children who kept cats compared with those in classes with fewer than 18% cat owners.[39] Thus passive exposure of school children to animal allergens can exacerbate asthma, even among children who are being treated with asthma medications. Experimental challenge studies carried out in a room containing cats have shown that airborne Fel d 1 levels of 10 to 100 ng/m^3 induced respiratory symptoms and caused changes in pulmonary function.[40,41] Similar airborne allergen levels are typically found in homes that contain cats. Somewhat paradoxically, two recent studies have shown that high-level exposure to Fel d 1 (> 20 μg/g) gives rise to a form of tolerance that results in a lower prevalence of IgE antibody responses.[42,43] This modified Th2 response is associated with production of high levels of Fel d 1–specific IgG4 antibody without IgE antibody.[42] In both studies, low-dose exposure to Fel d 1 (1 to 8 μg/g) was most strongly associated with the development of IgE antibody. The dose-response studies may explain why, in population surveys, sensitization to cats is often lower than that to dust mites. In countries such as New Zealand, where 78% of the population owns cats and high levels of allergen occur in houses, the prevalence of sensitization to cats is only 10% and cats is not as important a cause of asthma as dust mites.[44]

CR allergen exposure has been assessed by measuring Bla g 1 and Bla g 2, which cause sensitization on 30% to 50% and 60% to 80% of CR-allergic patients, respectively.[17] Most dust samples from CR-infested homes contain both allergens, although there is only a modest quantitative correlation between levels of the two allergens. Analysis of Bla g I and Bla g 2 levels in the homes of asthma patients admitted to the emergency departments in Atlanta, Georgia, and Wilmington, Delaware, showed that homes with visible evidence of CR contained more than 8 U/g Bla g 1 and more than 2 U/g (approximately 0.1 μg/g) Bla g 2.[45,46] In inner-city Baltimore, the proportion of asthmatic children (aged 4 to 9 years) with positive skin tests to CR increased from 32% among children exposed to 1 to 2 U/g Bla g 1 to 45% among children exposed to more than 4 U/g.[7] Multicenter case-control studies carried out

TABLE 25-2 Allergen Exposure Thresholds for Sensitization

	Allergen Level in Dust Sample				
*Risk for Sensitization**	*Mite Group 1 (μg/g)*	*Fel d 1 (μg/g)*	*Can f 1 (μg/g)*	*Bla g 1 (U/g)*	*Bla g 2 (μg/g)*
High	> 10	1-8	1-8	> 8	> 1
Medium	2-10	8-20	8-20	1-8	0.08-0.4
Low	< 0.3†	< 0.5	< 0.5	< 0.6	<< 0.08
		> 20	> 20?		

*For atopic children.

†Levels found in "allergen-free" hospital rooms or in houses/apartments maintained for at least 6 months are less than 45% relative humidity.

among 12- to 13-year-old school children in Charlottesville, Virginia, and Los Alamos, New Mexico, showed that a fourfold increase in Bla g 2 exposure (from 0.08 to 0.33 μg/g) was associated with highly significant increases in wheal size of CR skin tests.[18] These studies provide evidence for a dose-response relationship between CR allergen exposure and sensitization. The reported CR allergen levels are severalfold lower than those for either mite or animal allergens, suggesting that CR may be more potent in stimulating IgE responses. The National Cooperative Inner City Asthma Study (NCICAS) showed that sensitization and exposure to CR (> 8 U/g Bla g 1) were associated with increased asthma morbidity. Among inner-city children from eight U.S. cities enrolled in the study, 37% were allergic to CR, and hospitalizations, unscheduled medical visits, and days lost from school due to wheezing or asthma were strongly associated with CR allergen exposure.[7,16] In the United States, high CR allergen levels are associated with lower socioeconomic status, living in inner cities, and race (African-American or Hispanic). However, CR allergy is not an entirely urban problem. Suburban and rural homes, including trailer homes, that harbor high levels of CR cause sensitization and respiratory disease in these populations. Moreover, asthma is the most important public health problem caused by CR infestation of homes.

The measurement of exposure to fungal allergens has proved more difficult than for other indoor allergens. Numerous fungal allergens have been cloned, and sensitive and specific assays for major allergens, such as Alt a 1 and Asp f 1, have been developed (see Box 25-1). The problems with measuring these allergens are related to the biology of fungal growth in houses. Unless houses are heavily contaminated, most *Aspergillus* occurs as spores and the Asp f 1 allergen is produced only when the spores germinate. Similarly, although the Alt a 1 assay is quite sensitive and the allergen can be eluted from spores, laboratory experiments indicate that unless the spore counts are very high (>100,000/ml), they are unlikely to be detected in the assay.[47,48] Success in using polyclonal antibody–based ELISA for "total" fungal allergens has been reported.[48] However, the limitations of these assays are that they measure both allergens and nonallergenic fungal proteins and that they cannot be used to provide quantitative assessments of allergen exposure. Other markers of fungal growth in houses include β(1-3) glucans, ergosterol, extracellular polysaccharides, and volatile organic compounds.[22] Until more clearly defined and validated allergen tests become available, it seems likely that volumetric air sampling and measurement of spore counts and fungi cultured from air/dust samples will remain the standard approaches to assess fungal exposure.

For mite, animal, and CR allergens, there are clear data on exposure thresholds that result in sensitization. However, an association between allergen exposure and asthma symptoms is less clear, and for mite and CR allergens, there is a poor relationship between current exposure and symptoms. A seminal 12-year prospective study by Sporik et al[49] of children born to atopic patients showed that the development of asthma at age 11 was strongly associated with high levels of dust mite exposure (>10 μg/g) in the first year of life. A mite allergen level of 10 μg/g has been regarded as a risk level for symptoms based on this study and earlier seasonal variation and hospital emergency department admission studies.[6] However, the German MAS study found no association between mite allergen exposure and asthma in the prospective cohort followed through age 7.[36] The MAS study has several limitations: the reported mite allergen levels were very low compared with other countries and did not include a wide range of exposure (reviewed in an article by Sporik and Platts-Mills[44]). It has also been argued that the concept that there should be a direct relationship between allergen exposure and asthma is flawed because any association is entirely dependent on sensitization. Thus in Atlanta, there is no association between CR allergen exposure and asthma, unless the degree of sensitization is taken into account.[44]

Monitoring Allergen Exposure as Part of Asthma Management

Expert guidelines for asthma management, produced by the National Heart, Lung, and Blood Institute (NHLBI) and the American Academy of Allergy, Asthma and Immunology (AAAAI), recommend allergen avoidance as a primary goal of asthma management.[50,51] The guidelines recommend using patient histories and allergic sensitization as evidence of allergen exposure but do not include any environmental assessment. There are several flaws with this approach. Allergen levels in homes vary widely across the United States, depending on climate, geographic location, housing type and condition, and socioeconomic status. The National Allergen Survey reported that 22% of U.S. homes contain more than 10 μg/g mite allergen and illustrate that high mite allergen levels are a potential problem in many homes.[52] Conversely, many U.S. homes have low or undetectable allergen levels. For example, Der f 1 levels in Boston, Massachusetts, were 10-fold to 100-fold higher in single-family homes than in centrally heated apartments.[53] Thus marked variations in allergen exposure were demonstrated in a single U.S. city. Other observations show that inner-city homes located on the East Coast have lower mite allergen levels than those reported in the urban South.[7,17,45,54,55] Clinically relevant levels of cat allergen are found in homes that do not contain cats and in some schools. Finally, CR allergen is found in about 20% of homes that have no visible evidence of CR infestation.[46] The significance of these studies is that allergen exposure should not be assumed and that knowledge of allergen levels in the home is needed to provide objective advice about exposure and avoidance.

A detailed discussion of environmental control measures for reducing allergen levels is given in Chapter 22. Allergen measurements have been used to validate the efficacy of a variety of physical and chemical control methods, procedures, and devices, including mattress encasings, vacuum cleaner filters, acaricides, protein denaturants, detergents and carpet cleaners, and steam cleaning, humidity control, and air filtration systems. For example, the quality of mattress and pillow encasings was greatly improved by using microfine cotton fabrics, and the precise pore size of these fabrics for allergen exclusion was established by airflow experiments and allergen analysis by ELISA.[56] It is important that products and devices be tested for their effects on specific allergens so that allergists can make objective recommendations based on scientific and technical data and allergic patients and other consumers can verify claims made by manufacturers.

The availability of new rapid screening tests for indoor allergens will educate patients about the role of allergens in causing allergic disease. The importance of educating patients so that they can play a leading role in controlling their disease

has recently been emphasized.[26,57] The objectives of making exposure measurements are to show that in addition to having IgE reactivity to an allergen, the patient may be exposed to the relevant allergen at home. This information is expected to reinforce the link among allergen sensitization, exposure, and disease activity; enable informed decisions to be made about treatment options; and encourage implementation and compliance with intervention procedures (Box 25-2). Support for this concept comes from recent European studies involving 360 asthma/rhinitis patients, which showed that the use of an "indoor environmental technician" to visit homes resulted in greater compliance with avoidance measures and greater reductions in allergen levels.[58] In well-designed clinical studies in the United States, it was reported that only 20% to 30% of patients adhere to avoidance recommendations.[51] Adherence improved to about 50% with intensive clinic-based education. These studies emphasize the need for improving compliance if allergen avoidance is to be effective. The hypothesis that knowledge of allergen exposure can be used as an educational tool that will encourage compliance needs to be tested in outreach studies. If these studies are successful, they could form the basis of simple environmental monitoring and control procedures to treat asthma, with significant cost savings and benefits for public health.

BOX 25-2 THERAPEUTIC PRINCIPLES
Allergen Exposure Measurements in Clinical Practice

Provide objective assessments of current allergen exposure.

Educate patients about sensitization, exposure, and allergic disease.

Allow allergists or pediatricians to provide clear information about the efficacy of avoidance procedures or devices.

Reinforce advice about immunotherapy or avoidance.

Encourage compliance with avoidance procedures.

CONCLUSIONS

Children and adults in Western countries have adopted sedentary lifestyles and spend 90% of their time indoors, where they may be exposed to high levels of environmental allergens. Modern housing has low air exchange rates and results in high airborne allergen levels, especially animal allergens. Although these observations do not explain the increase in asthma prevalence that has occurred during the past 40 years, they may contribute to the increase in allergen sensitization seen in children. Epidemiologic studies worldwide have proved an association between allergen exposure and IgE-mediated sensitization and have consistently shown a strong association between allergen sensitization and asthma.[44] Allergen exposure can be accurately measured by ELISA for major allergens, which have been reported in more than 250 peer-reviewed publications. The gap in the repertoire is that there are few tests for fungal allergens; this has limited studies on the role of fungal allergen exposure in causing allergic disease and other respiratory conditions. Currently, measurement of allergen in reservoir dust samples provides the best index of allergen exposure in the home. An increasing array of semiquantitative tests is being developed for use in physicians' offices and by allergic patients. These tests should empower patients to monitor allergen levels in the home and to implement suitable avoidance procedures in consultation with allergists and allergy clinic staff. The next generation of allergy tests will enable multiple allergens to be tested on a dust or air sample at the same time and within 10 minutes, thus making allergen detection systems widely available for the consumer. Home allergen assessments will enable allergists to provide objective information about allergen exposure and will educate patients about allergic disease. This approach should encourage compliance with allergen avoidance and the implementation of procedures to reduce allergen levels. Environmental control is an integral part of asthma management and should be considered for children with atopic dermatitis. Prospective controlled trials are under way to establish whether the primary avoidance of allergens in infancy will reduce allergen sensitization and the prevalence of asthma. Preliminary results of a large cohort studied at 3 years show a reduced prevalence of wheeze among children on avoidance compared with control children.[59] The combination of improved allergen-monitoring techniques and validated allergen-avoidance procedures should enable low allergen conditions to be established in homes. This strategy should improve asthma management and reduce the public health problems associated with sensitization to indoor allergens.

HELPFUL WEBSITES

The WHO/IUIS Allergen Nomenclature Sub-committee website (www.allergen.org)
The National Institute for Environmental Health Sciences website (www.niehs.nih.gov)
The American Academy of Allergy Asthma and Immunology website (www.aaaai.org)
The American College of Allergy Asthma and Immunology website (www.acaai.org)
The Environmental Protection Agency website (www.epa. gov)
The National Institute for Allergy and Infectious Diseases website (www.niaid.nih.gov/research/dait.htm)
The Allergy Report website (www.theallergyreport.org/main.html)

REFERENCES

1. Kern RA: Dust sensitization in bronchial asthma, *Med Clin North Am* 5:751-758, 1921.
2. Cooke RA: Studies in specific hypersensitiveness, IV: new aetiologic factors in bronchial asthma, *J Immunol* 7:147-162, 1922.
3. Van Leeuwen SW: Asthma and tuberculosis in relation to "climate allergens," *Br Med J* 2:344-347, 1927.
4. Berrens L, Young E: Preparation and properties of purified house dust allergen, *Nature* 190:536-537, 1961.
5. Voorhorst R, Spieksma FthM, Varekamp H, et al: The house dust mite (*Dermatophagoides pteronyssins*) and the allergens it produces, *J Allergy* 39:325-339, 1967.
6. Platts-Mills TAE, Vervloet D, Thomas WR, et al: Indoor allergens and asthma: report of the Third International Workshop, *J Allergy Clin Immunol* 100:S1-S24, 1997.
7. Eggleston PA, Rosenstreich D, Lynn H, et al: Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma, *J Allergy Clin Immunol* 102:563-570, 1998.
8. Bush RK, Eggleston PA, eds: Guidelines for control of indoor allergen exposure, *J Allergy Clin Immunol* 107:S403-S440, 2001.
9. Peat JK: Can asthma be prevented? Evidence from epidemiological studies of children in Australia and New Zealand in the last decade, *Clin Exp Allergy* 28:261-265, 1997.

10. Custovic A, Taggart SCO, Francis HC, et al: Exposure to house dust mite allergens and the clinical activity of asthma, *J Allergy Clin Immunol* 98:64-72, 1995.
11. Chan-Yeung M, Manfreda J, Dimich-Ward H, et al: Mite and cat allergen levels in homes and severity of asthma, *Am J Respir Crit Care Med* 152:1805-1811, 1995.
12. Bollinger ME, Eggleston PA, Flanagan E, et al: Cat antigen in homes with and without cats may induce allergic symptoms, *J Allergy Clin Immunol* 97:907-914, 1996.
13. Custovic A, Green R, Fletcher A, et al: Aerodynamic properties of the major dog allergen Can f 1: distribution in homes, concentration, and particle size of allergen in the air, *Am J Resp Crit Care Med* 155:94-98, 1997.
14. Ingram JM, Sporik R, Rose G, et al: Quantitative assessment of exposure to dog (Can f 1) and cat (Fel d 1) allergens: relationship to sensitization and asthma among children living in Los Alamos, New Mexico, *J Allergy Clin Immunol* 96:449-456, 1995.
15. Phipatanakul W, Eggleston PA, Wright EC, et al: Mouse allergen, II: the relationship of mouse allergen exposure to mouse sensitization and asthma morbidity in inner-city children with asthma, *J Allergy Clin Immunol* 106:1075-1080, 2000.
16. Rosenstreich DL, Eggleston P, Kattan M, et al: The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma, *N Engl J Med* 336:1356-1363, 1997.
17. Arruda LK, Vailes LD, Ferriani VP, et al: Cockroach allergens and asthma, *J Allergy Clin Immunol* 107:419-428, 2001.
18. Sporik R, Squillace SP, Ingram JM, et al: Mite, cat, and cockroach exposure, allergen sensitisation, and asthma in children: a case-control study of three schools, *Thorax* 54:675-680, 1999.
19. Chapman MD, Smith AM, Vailes LD, et al: Recombinant allergens for diagnosis and therapy of allergic disease, *J Allergy Clin Immunol* 106:409-418, 2000.
20. Aalberse RC: Structural biology of allergens, *J Allergy Clin Immunol* 106:228-238, 2000.
21. Pomes A, Chapman MD, Vailes LD, et al: Cockroach allergen Bla g 2: structure, function, and implications for allergic sensitization, *Am J Respir Crit Care Med* 165:391-397, 2002.
22. Bush RK, Portnoy JM: The role and abatement of fungal allergens in allergic diseases, *J Allergy Clin Immunol* 107:S430-S440, 2001.
23. Shakib F, Schulz O, Sewell H: A mite subversive: cleavage of CD23 and CD25 by Der p 1 enhances allergenicity, *Immunol Today* 19:313-316, 1998.
24. Wan H, Winton HL, Soeller C, et al: Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions, *J Clin Invest* 104:123-133, 1999.
25. Hiller R, Laffer S, Harwanegg C, et al: Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment, *FASEB J* 16:414-416, 2002.
26. Chapman MD, Tsay A, Vailes LD: Home allergen monitoring and control: improving clinical practice and patient benefits, *Allergy* 56:604-610, 2001.
27. Tsay A, Williams L, Mitchell EB, et al: A rapid test for detection of mite allergens in homes, *Clin Exp Allergy* 32:1596-1601, 2002.
28. Polzius R, Wuske T, Mahn J: Wipe test for the detection of indoor allergens, *Allergy* 57:143-145, 2002.
29. Custovic A, Taggart SCO, Niven RM, et al: Evaluating exposure to mite allergens, *J Allergy Clin Immunol* 96:134-135, 1995.
30. Luczynska CM, Li Y, Chapman MD, et al: Airborne concentrations and particle size distribution of allergen derived from domestic cats (*Felis domesticus*). Measurements using cascade impactor, liquid impinger, and a two-site monoclonal antibody assay for Fel d I, *Am Rev Respir Dis* 141:361-367, 1990.
31. Ehnert B, Lau-Schadendorf S, Weber A, et al: Reducing domestic exposure to dust mite allergen reduces bronchial hyperreactivity in sensitive children with asthma, *J Allergy Clin Immunol* 90:135-138, 1992.
32. de Blay F, Sanchez J, Hedelin G, et al: Dust and airborne exposure to allergens derived from cockroach (Blattella germanica) in low-cost public housing in Strasbourg (France), *J Allergy Clin Immunol* 99:107-112, 1997.
33. O'Meara T, Tovey ER: Monitoring personal allergen exposure, *Clin Rev Allergy Immunol* 18:341-395, 2000.
34. Custis N, Woodfolk JA, Vaughan JW, et al: Use of a novel air cleaner to monitor airborne allergen, *J Allergy Clin Immunol* 109:S53, 2002.
35. Squillace SP, Sporik RB, Rakes G, et al: Sensitization to dust mites as a dominant risk factor for asthma among adolescents living in central Virginia. Multiple regression analysis of a population-based study, *Am J Respir Crit Care Med* 156:1760-1764, 1997.
36. Lau S, Illi S, Sommerfeld C, et al: Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicentre Allergy Study Group, *Lancet* 356:1392-1397, 2000.
37. Custovic A, Green R, Taggart SC, et al: Domestic allergens in public places, II: dog (Can f 1) and cockroach (Bla g 2) allergens in dust and mite, cat, dog and cockroach allergens in the air in public buildings, *Clin Exp Allergy* 26:1246-1252, 1996.
38. Patchett K, Lewis S, Crane J, et al: Cat allergen (Fel d 1) levels on school children's clothing and in primary school classrooms in Wellington, New Zealand, *J Allergy Clin Immunol* 100:755-759, 1997.
39. Almqvist C, Wickman M, Perfetti L, et al: Worsening of asthma in children allergic to cats, after indirect exposure to cat at school, *Am J Respir Crit Care Med* 163:694-698, 2001.
40. Wood RA, Eggleston PA. Environmental challenges to animal allergens. In Spector S, ed: *Provocation testing in clinical practice,* New York, 1994, Marcel Dekker.
41. Wood RA, Eggleston PA: The effects of intranasal steroids on nasal and pulmonary responses to cat exposure, *Am J Respir Crit Care Med* 151:315-320, 1995.
42. Platts-Mills T, Vaughan J, Squillace S, et al: Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study, *Lancet* 357:752-756, 2001.
43. Custovic A, Hallam CL, Simpson BM, et al: Decreased prevalence of sensitization to cats with high exposure to cat allergen, *J Allergy Clin Immunol* 108:537-539, 2001.
44. Sporik R, Platts-Mills TAE: Allergen exposure and the development of asthma, *Thorax* 56(suppl II):II58-II63, 2001.
45. Call RS, Smith TF, Morris E, et al: Risk factors for asthma in inner city children, *J Pediatr* 121:862-866, 1992.
46. Gelber LE, Seltzer LH, Bouzoukis JK, et al: Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital, *Am Rev Respir Dis* 147:573-578, 1993.
47. Vailes LD, Sridhara S, Cromwell O, et al: Quantitation of the major fungal allergens, Alt a 1 and Asp f 1 in commercial allergenic products, *J Allergy Clin Immunol* 107:641-646, 2001.
48. Barnes C, Tuck J, Simon S: Allergenic materials in the house dust of allergy clinic patients, *Ann Allergy Asthma Immunol* 86:517-523, 2001.
49. Sporik R, Holgate ST, Platts-Mills TA: Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood: a prospective study, *N Engl J Med* 323:502-507, 1990.
50. National Asthma Education and Prevention Program, Expert Panel Report 2. *Guidelines for the diagnosis and management of asthma,* Bethesda, MD, 1997, National Institutes of Health, National Heart, Lung, and Blood Institute. NIH Pub No. 97-4051.
51. American Academy of Allergy, Asthma and Immunology: Position statement. Environmental allergen avoidance in allergic asthma, *J Allergy Clin Immunol* 103:203-205, 1999.
52. Vojta PJ, Friedman W, Marker DA, et al: First National Survey of Lead and Allergens in Housing: survey design and methods for the allergen and endotoxin components, *Environ Health Perspect* 110:527-532, 2002.
53. Chew GL, Higgins KM, Gold DR, et al: Monthly measurements of indoor allergens and the influence of housing type in a northeastern US city, *Allergy* 54:1058-1066, 1999.
54. Miller RL, Chew GL, Bell CA, et al: Prenatal exposure, maternal sensitization, and sensitization *in utero* to indoor allergens in an inner-city cohort, *Am J Resp Crit Care Med* 164:995-1001, 2001.
55. Nelson RP Jr, DiNicolo R, Fernandez-Caldas E, et al: Allergen-specific IgE levels and mite allergen exposure in children with acute asthma first seen in an emergency department and in non asthmatic control subjects, *J Allergy Clin Immunol* 98:258-263, 2001.
56. Vaughan JW, McLaughlin TE, Perzanowski MS, et al: Evaluation of materials used for bedding encasement: effect of pore size in blocking cat and dust mite allergen, *J Allergy Clin Immunol* 103:227-231, 1999.
57. Platts-Mills TA, Vaughan JW, Carter MC, et al: The role of intervention in established allergy avoidance of indoor allergens in the treatment of chronic allergic disease, *J Allergy Clin Immunol* 106:787-804, 2000.
58. deBlay F, Fourgaut G, Lieutier-Colas F, et al: Mite allergen reduction: role of an indoor technician in patients' compliance and allergen exposure, *J Allergy Clin Immunol* 107:S218, 2001.
59. Custovic A, Simpson BM, Simpson A: Effect of environmental manipulation in pregnancy and early life on respiratory symptoms and atopy during first year of life: a randomised trial, *Lancet* 358:188-193, 2001.