

Allergens

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An increase in the prevalence and severity of asthma has been observed in developed countries throughout the world. Concurrently, societal changes have been complex, inter-related, and far reaching. The shift toward single-parent families and families in which both parents work has led to an increased number of children in daycare. At the same time, childhood vaccinations have changed the incidence of common childhood diseases. With parents spending less time at home or perhaps questioning their children's safety outside and with the pervasiveness of television in our culture, children spend far more time indoors. Home environments have changed, resulting in differences in allergen exposure. Furthermore, exposure to some pollutants, diesel particulates, and ozone has increased. Given the complexity of the interactions, it is likely that a combination of factors has resulted in the overall increase in disease prevalence and severity that has been observed.

Sensitization to allergens found indoors, such as dust mite, cat, and cockroach, is a risk factor for asthma. As molecular knowledge of allergens becomes greater and our understanding of the immune system increases, differences in allergens and in responses to them have become apparent. In the past, attempts to evaluate the role of allergens in asthma looked to knowledge of dust mite as the model. It may not be that simple; we do not believe that the tendency to develop IgE ab is the same regardless of the allergen. It also may be that allergens affect the respiratory or immune systems directly to cause wheezing. In this article, current information is presented on the immune response to dust mite, pets, cockroach, and *Alternaria*, and thus their role as asthma triggers.

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Dust mite

As the major allergen associated with atopic asthma, dust mites have been the center of much research. Dust mites are arthropods, but being in the order *acari* they are not closely related to insects [1]. They acquire water from water vapor in the environment. Thus, dust mite growth varies throughout the world depending on the climate. There is virtually no measurable dust mite allergen present in the frigid (and consequently dry) areas of northern Sweden, whereas some of the highest levels are found in New Zealand. Between those extremes, a wide range of levels is found in the United States, United Kingdom, and South America. In temperate areas, the prominent species are *Dermatophagoides pteronyssinus* and *D. farinae*. A study of eight regions within the United States determined that most homes have both species, with the dominant species varying within homes in each region [2]. *Euroglyphus maynei* was found in the most humid, southern regions. In tropical and subtropical regions, *Blomia tropicalis* is clinically important. Many different dust mite allergens have been characterized. Recently Der f 18, a 60-kD protein with chitinase homology, was purified and cloned [3]. Those most extensively studied are the group 1 and 2 allergens, a 25-kD cysteine protease and 14-kD epididymal protein, respectively (Table 1) [4,6–8]. Several other mite allergens are enzyme proteases secreted in mite feces. Group 10 allergens (Der p 10 and Der f 10) are of interest because they are tropomyosins that may lead to IgE ab cross-reactivity between mites and other invertebrates, such as shrimp and cockroaches [9].

Table 1
Properties of allergens associated with asthma

Allergen	Molecular weight (kD)	Function (homology)	Mode of exposure
Mite			
Der p 1	25	Cysteine protease	Fecal particles
Der p 2	14	(epididymal protein)	
Der p 9	24	Serine protease	
Der p 10	36	Tropomyosin	
Cat			
Fel d 1	36	Uteroglobulin	Sebaceous glands
Fel d 2		Albumin	
Dog			
Can f 1	25	Lipocalin	
Cockroach			
Bla g 1		(Mosquito protein)	Saliva
Bla g 2	36	Aspartic protease	Fecal particles
Bla g 4	21	Lipocalin	
Bla g 5	22	Glutathione transferase	
Per a 7	37	Tropomyosin	
Alternaria			
Alt a 1	28	Unknown	Spores
Rye grass			
Lol p 1	27	Unknown	Pollen

Data from Refs. [4,52] and www.allergen.org.

Longitudinal studies have confirmed the relevance of the relationship between allergy to house dust mite and asthma. Recently published results of a large population-based birth cohort in New Zealand revealed that sensitivity to dust mite (by skin test results) was associated with persistent symptoms and relapse of asthma in adulthood [10]. The 21.2% who reported wheezing at only one assessment had a markedly increased prevalence of sensitivity to dust mite. Nonetheless, atopy seems to be a major determinant of continued wheeze.

It remains confusing that many children are exposed and exhibit evidence of sensitivity to dust mite but do not report symptoms. Several groups have examined the relationship between dust mite exposure and incident asthma. In a group of children at risk due to family history, Sporik et al [11], with a geometric mean exposure level of 18.4 $\mu\text{g/g}$ mattress dust in 1979 and 10.6 $\mu\text{g/g}$ in 1989, showed a strong correlation between wheezing and sensitization to dust mite (relative risk of asthma of 19.7 in a child sensitized to dust mite). Furthermore, there was a correlation between early high exposure to dust mite and wheezing. By contrast, in Germany, with mean exposure to dust mite up to 5.6 $\mu\text{g/g}$, wheezing was strongly associated with sensitivity to mite (30% of wheezing children), but a dose-response relationship between mite exposure and wheezing was not evident [12]. In a recent study in the United States in which the geometric mean concentration of mite allergen was 2.8 $\mu\text{g/g}$ and only 19% of children were exposed to $> 10 \mu\text{g/g}$ of dust, there was no evidence that high levels of exposure were associated with increased risk of asthma [13].

In addition to a high prevalence of IgE ab to mite, there is evidence that the titer of IgE ab to mite can be markedly elevated. From the standpoint of the general ability of the dust mite allergen to induce the production of IgE ab, the highest IgE ab responses in patients with atopic dermatitis (AD) were observed to dust mite [14]. We have also observed high titers of IgE ab to mite among children living in New Zealand. In children with and without wheezing, the geometric mean titer of IgE ab to mite was 22.1 IU/mL. This IgE ab response to mite comprised up to 20% of the total IgE measurement in $> 15\%$ of the children with wheezing. These observations may be consistent with *in vitro* evidence that Der p 1 has the ability to cleave CD23 [15]. One isoform of the CD23 molecule is found on activated B cells and can function as an Fc receptor, binding IgE and interacting with CD21. Through these roles, it may have an immune regulatory function over IgE ab production. At high levels, IgE ab binds CD23, causing feedback inhibition of further IgE synthesis. Thus, a decrease in surface expression of CD23 by dust mite allergen could be viewed as "proallergic." The ability of Der p 1 to effect cleavage of CD23 would be expected to be inhibited by normal function of respiratory antiproteases, but patients with asthma may have reduced defenses due to inflammation.

Immunologically, asthma is viewed as a complex disease that results from a combination of IgE-mediated and cell-mediated mechanisms. Dust mite particles (and allergen) have been shown to have non-IgE-mediated direct inflammatory action. Specifically, nitric oxide (NO), which is elevated in the airways of patients with asthma, has been shown to increase in rat alveolar macrophages exposed to

dust mite fecal particles or Der p 1 and Der p 2 [16]. The concentration of NO produced was dose dependent and time dependent, with maximal production at 48 hours, which is consistent with persistent inflammation in response to exposure that is observed clinically. The mechanism was believed to be through induction of iNOS.

Respiratory epithelial cells have been shown to release inflammatory mediators. The allergens Der p 1 and Der p 9 (cysteine and serine proteases, respectively) were shown to directly stimulate release of granulocyte/macrophage colony-stimulating factor, interleukin (IL)-6, and IL-8 from human bronchial epithelial cells (in transformed and freshly cultured cells) [17]. The action of the proteases was dose dependent, with significant cytokine release at 8 hours. Only 30 minutes of exposure was necessary, and the response was inhibited by antiproteases.

Pet paradox

Reviewing the evidence for a relationship between pet ownership and prevalence and symptoms of asthma presents a contrast with the evidence in relation to dust mites. Specifically, the immune response to cat exposure is markedly different from the dose-dependent development of sensitization observed to dust mite. The underlying concept is that high exposure to cat allergen results in a lower prevalence of sensitization to cat. This observation was initially presented by a group of investigators in Sweden [18]. It was confirmed in several areas throughout the world, including the United States, the United Kingdom, northern Sweden, and New Zealand (Table 2) [19–21]. In the initial report of Hesselmar et al [18], pet exposure during the first year of life was associated with decreased allergic diagnoses, including allergic rhinitis, asthma, and cat sensitization.

Table 2
Studies that show reduced risk of sensitization and asthma among children living with a pet

Study	Region	Allergen		Sensitization		Asthma
		Cat	Dog	Specific	Nonspecific	
Hesselmar et al [18]	Sweden	√	√	√		√
Roost et al [72]	Europe/ New Zealand/Australia	√		√		
Platts-Mills et al [19]	Los Alamos, NM/ Charlottesville, VA	√		√		√
Remes et al [73]	Tucson, AZ		√			√
Custovic et al [20]	United Kingdom	√		√		
Litonjua et al [29]	Boston, MA	√	√			√
Perzanowski et al [21]	Northern Sweden	√			√	√
Ownby et al [22]	Detroit, MI		√		√	
Svanes et al [74]	Europe/New Zealand/Australia		√		√	
Ronmark et al [66]	Northern Sweden	√	√		√	
Gern et al [23]	Madison, WI		√		√	

Eczema and sensitization to other allergens tested were not decreased with early pet exposure. The lower prevalence of asthma (odds ratio [OR] 0.34 in those who lived with a cat) was not explained by selection bias due to family history of allergy. A careful review of their results reveals several related issues that have emerged, including the importance of timing of pet exposure and the possibility of selection bias of patients, with a family history of allergy, against keeping pets. In this study, the relationship between pet ownership and asthma remained significant even when families who chose not to own a pet because of family history of allergy were excluded [18]. In addition, family history did not influence the results seen in Detroit or the United Kingdom [20,22].

The next question is whether exposure to dogs has the same effect on the immune system and the development of allergic sensitization and asthma. In Detroit, a decreased prevalence of allergic sensitization to any allergen was observed among children who were living with two or more dogs or cats [22]. Similarly, in Wisconsin it was observed that children living with dogs were significantly less likely to have a positive RAST test result at 1 year of age [23]. In these studies, cat exposure did not decrease sensitization in general. Almqvist [24] found that dog exposure early in life was associated with a decrease in sensitization to other allergens and a decrease in asthma.

The contrast in responses to cat and dog introduces a further question about the mechanism underlying the relationship between pet ownership and allergic response: Is it a shared response or allergen specific? One theory has focused on the role of endotoxin, a component of bacterial cell walls. The idea came from observations of decreased atopy and, in some studies, wheezing among children growing up on farms [25–27]. Bräun-Fahrlander et al [28] measured endotoxin levels in rural Germany and found that exposure to increased amounts of endotoxin was associated with decreased atopic sensitization. The relationship between endotoxin exposure and wheezing was dependent upon the type of wheezing. Only atopic wheezing was reduced in association with high endotoxin levels. Others have shown that exposure to endotoxin was associated with increased risk of wheezing in early life [29]. Alternatively, it has been proposed that cats induce an immune-specific response characterized by the production of IgG ab without IgE ab. Because this IgG was largely of the IgG₄ subclass (which is IL-4 dependent), this has been termed the modified Th2 response [19]. Investigation of T-cell responses further supported the concept of specific immune control because allergic and “tolerant” individuals exhibited increased levels of IL-10 in cultures stimulated with peptides of Fel d 1 [30].

The major allergen in cat dander, Fel d 1, was isolated in 1974 [31]. It is found in saliva and sebaceous secretions. Purification and measurement became possible with the development of monoclonal antibodies in 1994 [32,33]. It was found to predominate over cat albumin in terms of prevalence of IgE ab in the sera of allergic individuals [34]. The crystal structure of the molecule, formed by two heterodimers, was recently characterized [35]. In so doing, the similarity of the tertiary structure of the molecule to that of the secretagogue uteroglobin was confirmed. This similarity suggests that Fel d 1 may share some of the abilities of

uterglobin to influence the immune response [36]. By contrast, the major dog allergen, Can f 1, is a lipocalin similar to those of other animals, including mouse (Mus m 1) and rat (Rat n 1).

In conclusion, pets present a paradox immunologically because cat allergen is a significant risk factor for asthma in the sensitized individual, but exposure does not result in dose-dependent sensitization. There is strong evidence to the contrary; high exposure to cat and dog allergen results in decreased sensitization. However, the underlying process seems to be different. Although high exposure to cat is specifically associated with decreased allergy to cat, the effect of high exposure to dog seems to be nonspecific. Although it is premature to say that all children should have pets, this knowledge must influence the recommendation allergists have made for many years that allergic children should not have pets.

Other animals

In the United States, attention has recently turned toward an assessment of the significance of other, sometimes unwanted, animals found in homes, including mice and rats. In the laboratory, a dose-response association has been observed to the allergens of these animals. Atopic workers were more likely to develop animal allergy with increased exposure [37]. In a subsample from the National Cooperative Inner-City Asthma Study (NCICAS), almost all of the homes studied (95%) had measurable mouse allergen in at least one room [38]. Examination of the children living in those homes revealed that 18% were sensitized to mouse, with high levels of exposure in the kitchen and other allergy as risk factors [39]. In that study, sensitization to mouse was not significantly related to asthma morbidity. Another study of the Baltimore area that included children with asthma living in city or suburban homes found a dose-response relationship between exposure and sensitization but did not directly relate exposure and symptoms [40]. In a recent analysis of NCICAS data for rat exposure, fewer homes (33%) had measurable rat allergen in any room by comparison to mouse allergen [41]. However, the prevalence of sensitization to rat (21%) was slightly higher than that to mouse. Although sensitization to rat was not significantly related to exposure, children who were sensitized and exposed to rat allergen were more likely to be hospitalized for asthma.

Cockroach

One area known for a high prevalence of severe asthma is inner-city areas of the United States. This argues against improved hygiene as a cause for increases in the disease. Thus, allergen exposure provides an appealing explanation in these areas. A correlation between cockroach sensitization and asthma has also been reported in Europe and Brazil and in suburbs in the United States [42–45]. Studies of emergency departments have shown an association between cockroach

allergy and severe asthma [46,47]. Rosenstreich's analysis of inner-city children strengthened evidence for the cockroach allergen effect by showing that the combination of exposure and allergic sensitization increased the risk of hospitalization [48]. They found a high prevalence of sensitization to cockroach (37%), dust mite (35%), and cat (23%); however, high levels of exposure were found primarily to cockroach (50% of houses). Furthermore, cockroach-allergic children with exposure had increased rates of hospitalization, wheezing, and missed school. Litonjua et al [49] reported a dose-response relationship between cockroach exposure and asthma in children living in Boston. Exposure to 2 U Bla g 1 or Bla g 2/g dust was associated with a high risk (OR >10) for developing asthma.

Appreciation of the significance of cockroach allergy is not new. Bernton and Brown [50] reported sensitization to cockroach and noted an increased prevalence (58%) in urban areas. Kang [51] showed a relationship between sensitization and asthma when she performed bronchial provocation testing with cockroach antigen. A significant decrease in pulmonary function occurred in 88% of sensitized patients, whereas none of the asthmatics who were not sensitized to cockroach reacted.

The major species infesting United States homes is German cockroach (*Blattella germanica*), whereas the American cockroach (*Periplaneta americana*) is more common in tropical areas such as Taiwan and Brazil. Initial studies to characterize the major allergens revealed two molecules, Cr-I and Cr-II (25.5 and 69 kD, respectively), to which 70% of sensitive patients exhibited positive skin tests [52]. In that early study, results were similar for American and German species. More recently, additional allergens have been described primarily from German cockroach. The function of Bla g 1 is unknown; it has an unusual structure containing tandem repeats [5]. Typically, the cockroach allergens do not seem to be cross-reactive between the two species, but Bla g 1 and Per a 1 are exceptions. Unlike some dust mite allergens, the allergens of cockroach are not active enzymes. Bla g 2 is an inactive aspartic protease resembling mammalian pregnancy-associated glycoproteins believed to function as binding proteins [53]. Bla g 4 is a lipocalin, as are many of the animal allergens, and Bla g 5 is a glutathione transferase [52]. Per a 7 was recently identified and has homology with tropomyosins, especially those in other invertebrates, such as shrimp and dust mite [54]. Bla g 1 and Bla g 2 are most frequently measured for exposure data.

In spite of the difficulty in isolating and identifying enzymatically active cockroach allergens, cockroach extract has been shown to have in vitro proteolytic effects [55]. Cockroach extract increased IL-8 production through a G-protein receptor. In addition to regulation of cytokines like IL-8, activation of this specific G-protein may influence airway function as evidenced through action on guinea pig smooth muscle.

Alternaria

Alternaria is unique among the important allergens associated with asthma in that it is primarily considered to be an outdoor exposure. Initial search for a major

allergen in *Alternaria* extracts showed a carbohydrate-rich fraction with RAST binding in >80% of sensitized individuals, ALT-I [56]. These results were observed by several other investigators who isolated a 29- to 31-kD fraction termed Alt a 1 [57]. Studies of *Alternaria* revealed that allergen measurements were extremely sensitive to culture conditions and extraction techniques [58]. Little is known about dose response to mold allergen. It has been shown that Alt a 1 is increased in germinated spores [59]. Outdoors, a warm, dry climate has been associated with increased levels of *Alternaria*. Recently, standards for indoor measurement have been proposed; however, studies to confirm their effectiveness are lacking. Thus, measurements of indoor exposure are limited.

In stark contrast to the paucity of biochemical data, evidence that *Alternaria* is clinically significant in relation to asthma is abundant. O'Hollaren et al [60] at the Mayo Clinic reported that patients with positive skin test to *Alternaria* had an increased risk of severe or fatal attacks. Subsequently, it became clear from cross-sectional studies that sensitization to *Alternaria* was a major risk factor for wheezing in Arizona and New Mexico [61,62].

Alternaria has been shown to induce increased quantities of specific IgE ab among subjects with atopic dermatitis and asthma. Using Pharmacia CAP to measure IgE ab, the mean quantity of IgE ab to *Alternaria* in those with AD and asthma was 4.1 IU/mL and 3.7 IU/mL, respectively; both were significantly higher than the control group [13].

Some of the inflammatory effects of fungi may be antibody independent. The cell walls of fungi, which contain β -glucans and polysaccharide (glucomannan), must have an effect on the immune system. However, most of the research has examined the influence of *Aspergillus* and has not focused on the inflammatory effects seen in asthma. In addition to observation of inhibition of the mucociliary system by *Aspergillus*, Wang et al [63] suggested that *Aspergillus* hyphae may activate monocytes through toll-like receptor-4 (TLR-4) and CD14.

Grass

Similar to molds, exposure to grasses occurs primarily outdoors and in most population-based or cohort studies has not been found to be an independent risk factor for asthma. Nonetheless, ragweed pollen or grass pollen exposure has been shown to be a dramatic trigger for asthma symptoms in areas where pollen counts are very high. In 1986, during a 1-week period in May when rye grass pollen counts reached >2000 counts per cm², 205 patients were treated for acute asthma at Travis Air Force Base in northern California [64]. This was 40% of the total number of patients seen for asthma during the period from July 1985 to June 1986. The patients with asthma were significantly more likely to be sensitized to grass pollen than control subjects. The specific IgE ab levels were markedly elevated. In this study, grass pollen levels were measured inside the homes of a subset of patients. All of the homes measured had >2 μ g Lol p 1/g dust, and most

(13/15) had $> 10 \mu\text{g}$ Lol p 1/g dust, providing evidence that sufficient quantities could be found indoors during the pollen season.

Summary

Given that children and adults spend little time outdoors, it is not surprising that sensitization to allergens found indoors is common. It may well be the fact that exposure to these allergens persists year round that explains their importance in relation to bronchial hyper-reactivity and asthma symptoms. Understanding the increase in asthma is more complex because this may reflect increased concentrations of allergens indoors, increased time spent indoors, and changes in immune response. However, the risk for asthma is consistently related to sensitization to one or more of the perennial allergens. In general, this risk relates to the presence of high concentrations of the allergens in the community [4,65]. Thus, for dust mites, cockroach, or rodent urinary allergens, there are good studies showing that sensitization is rare in communities where the allergens are not present [61,62,66]. The recent evidence that sensitization is not increased by the presence of a cat or a dog has added considerable confusion primarily because the results from different studies are not the same (Table 2). It is not resolved whether the effect of animals (1) requires exposure early in life, (2) is nonspecific (ie, reduces sensitization to other allergens), or (3) can be explained by family choice (this is unlikely). The most recent evidence argues that exposure to cats in childhood can produce an allergen-specific form of tolerance that controls the prevalence of sensitization and the titer of IgE ab among sensitized children [30,67].

The evidence about cat argues strongly that all allergens are not created equal. This implies that there is a biologic or immunologic difference between the response to dust mite and cat allergens (Table 3). Whether other allergens can be classified is not clear, but the preliminary evidence suggests that cockroach and pollen allergens may be comparable to dust mite (ie, Class I), whereas dog and rodent allergens may induce tolerance (ie, Class II). There is another consequence of the results that may be equally relevant to understanding the role of allergens

Table 3
Biologic and immunologic differences between dust mite and cat allergens

	Class I	Class II
Source	Dust mite (cockroach, grass)	Cat (dog)
Particles	10–30 μm (fecal)	2.5–20 μm
Airborne	Airborne only with disturbance	Remain airborne
Nature of protein	Enzymatic potential: known to cleave CD23, CD25, and open tight junctions	Not enzymes: homology with uteroglobin (known immunomodulator) by sequence and structure
Immune response	No evidence for tolerance, includes high titer of IgE ab	Induces tolerance at high exposure, lower titers of IgE ab

in asthma. The presence of IgE ab to cat (or dog) is strongly associated with asthma regardless of the exposure levels [68]. Thus, it seems clear that it is the IgE ab, not IgG ab, IgG₄ ab, or T cells, that creates the risk of asthma [69]. It is important to recognize that all the evidence about the association between allergens and asthma relates to immediate skin tests or serum IgE ab. At a simple level it could be argued that this implies an immediate relationship between allergen exposure and asthma that is comparable to an immediate challenge response. However, there is plenty of evidence that the response is delayed in time course and that the effect of prolonged exposure persists for weeks or months even in an allergen-free environment. Also, there is evidence that T cells are involved. Thus, the correct conclusion is that IgE ab plays a critical role in the initial development of symptoms or in establishing inflammation of the lungs. A possible scenario would be that sensitization of the lungs with IgE ab would allow the local recruitment of specific T cells, which contribute to prolonged inflammation in the lungs.

Attempts to establish that allergen exposure correlated directly with acute episodes of asthma have not been convincing. Many attacks occur during a period when the patient has had continuing high exposure, but only a minority can be ascribed to a recent increase in exposure. Thus, the primary triggers of symptoms are nonspecific factors, such as cold air, exercise, passive smoke, etc. In most cases these triggers are acting on lungs that have been inflamed by chronic, perennial, low-grade allergen exposure. Similarly, a major cause of moderate or severe episodes of wheezing is a rhinovirus infection in a patient with underlying allergen-induced inflammation [70]. The situation with rhinovirus infection is striking because multiple studies have shown that rhinovirus challenge has an effect only on the lungs of allergic individuals. Among children and adults presenting to hospital with asthma, it is the combination of allergy and positive polymerase chain reaction for rhinovirus that is associated with the strongest risk [71].

Summary

The association between allergen sensitization and asthma is so strong that it is inevitable that allergens play a role in the disease. This conclusion is strongly supported by a wide range of experiments where patients are removed from their houses. Further, it is the allergens found in homes of the community that are relevant to asthma. On the other hand, the detailed triggers of attacks come from many different nonspecific factors acting on an inflamed lung.

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