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Cockroach allergens and asthma

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Asthma and allergy are the most common diseases associated with cockroach infestation of houses in the United States and other parts of the world. Sensitization and exposure to cockroach allergens is associated with increased asthma morbidity in the United States, especially among lower socioeconomic groups, including African American and Hispanic populations. Exposure to cockroach allergens in the first 3 months of life has been associated with repeated wheezing and asthma. The principal domestic cockroach species are *Blattella germanica* and *Periplaneta americana*. Both species produce several potent allergens, including Bla g 2 (inactive aspartic proteinase), Bla g 4 (calycin), Bla g 5 (glutathione-S-transferase), the group 1 cross-reactive allergens Bla g 1 and Per a 1, and tropomyosin. Structural homology between tropomyosins from cockroaches, mites, and shrimp may explain clinical cases of the oral allergy syndrome. The 3-dimensional structures of several cockroach allergens are known, and biologically active recombinant allergens have been produced in high-level expression vectors. The use of recombinant cockroach allergens should allow mechanisms of cockroach-induced asthma to be investigated and may lead to the development of new approaches to asthma treatment. Environmental allergen measurements of Bla g 1 and Bla g 2 have allowed exposure levels that cause allergic sensitization to be established. Abatement studies have shown that a sustained decrease in cockroach allergen levels is difficult but can be accomplished by professional application of insecticides, together with rigorous household cleaning. Cockroach asthma is an important public health problem that affects patients who are the least likely to be compliant with treatment with asthma medications or environmental control. Patient education, improvements in the housing stock, and improvements in environmental and immunologic treatment strategies are likely to be the most successful approaches to reduce the prevalence of cockroach-induced asthma. (J Allergy Clin Immunol 2001;107:419-28.)

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Cockroach allergy has been recognized as an important cause of asthma for over 30 years. In 1964, Bernton and Brown[1] were the first to report positive skin test responses to cockroach allergen (44%) in a landmark study of 755 allergy clinic patients living in New York. They also observed that 13% of patients who otherwise would have been considered nonatopic were sensitized to cockroach allergen alone. Subsequently, Mendoza and Snyder[2] and Schulaner[3] reported a high incidence of cockroach sensitivity in children from New York and showed that cockroach sensitivity was associated with low socioeconomic status. Bernton et al[4] performed bronchial challenges in 10 patients using cockroach extract and showed that they all had immediate responses. Subsequently, Kang et al[5] confirmed the causal relationship between cockroach allergy and asthma by showing early, late-phase, and dual bronchoconstriction after inhalation of cockroach extract by sensitized asthmatic patients. They also noted a significant increase in peripheral blood eosinophils 24 to 48 hours after challenge. These early studies demonstrated that asthma caused by cockroach allergen was antigen specific and similar to other types of atopic asthma. In the United States the prevalence of cockroach allergy ranges from 17% to 41% in various studies involving both children and adults.[6-13] Asthma and allergy are the most common diseases attributable to cockroach infestation of housing and are an important public health problem. Sensitization and exposure to cockroach allergen is associated with increased asthma morbidity in children living in the inner cities of the United States.[8,9,11,12] However, cockroach-induced asthma occurs whenever substandard housing permits cockroach infestation. This includes rural and semirural areas, suburbs, and small towns and cities across the United States.[6,10]

Cockroaches have been reported to be associated with asthma in many regions of the world, including Taiwan, Japan, Thailand, and Singapore in the Pacific Rim; Costa Rica and Puerto Rico in Central America; India; South Africa; and parts of Europe.[14] Recently, we have shown

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Abbreviation used
GST: Glutathione-S-transferase

that 55% of children and young adults with asthma, rhinitis, or both living in Brazil had positive skin test responses to cockroach allergen.[15] The most common domiciliary cockroach species are *Blattella germanica* (German cockroach) and *Periplaneta americana* (American cockroach). *B germanica* is a small cockroach, approximately three quarters of an inch in length, that commonly infests houses in the United States. *P americana* is a large cockroach, approximately 2 inches in length, that infests houses, schools, hospitals, and other large buildings. American cockroaches are less fertile than German cockroaches, require higher temperatures (~80°F) and humidity for optimal population growth, and are the predominant species in tropical countries, including Taiwan and Brazil.[14,15]

Over the last 10 years, several cockroach allergens have been identified, sequenced, and produced as biologically active recombinant proteins.[14] However, commercial cockroach extracts used for diagnosis and treatment remain nonstandardized, and well-designed controlled studies investigating the role of specific immunotherapy in the treatment of patients with cockroach allergy are lacking. Strategies for decreasing environmental exposure to cockroach allergen in inner-city homes have recently been investigated. The results suggest that a sustained decrease in cockroach allergen levels is difficult to accomplish, even after successful extermination of cockroach populations.[16–18]

This review will focus on recent studies on the role of exposure to cockroach allergens in the development of asthma and on structural aspects of cockroach allergens, which may influence the immune response to cockroach allergen in allergic patients. The reported association of early exposure to cockroach allergens and subsequent development of recurrent wheezing and asthma is intriguing and is considered in light of the current *hygiene hypothesis*. The use of recombinant cockroach allergens should allow mechanisms of cockroach-induced asthma to be further investigated and may lead to the development of new approaches for the treatment of asthma in the future.

COCKROACH ALLERGEN EXPOSURE AND SENSITIZATION

Although cockroach allergens are found throughout the house, including beds, furniture, and carpets, the highest levels are typically found in the kitchen, and these levels are perhaps the best indicator of cockroach infestation in a house.[8,14,19] However, exposure in the bedroom and family room may be more relevant in causing sensitization. Results of the National Cooperative Inner City Asthma Study revealed that the combination of sensitization and exposure to cockroach allergens was a risk factor for severity of asthma in children from large cities in the United States.[12] In that study exposure to Bla g 1 levels of greater than 8 U/g in children's bedrooms was strongly associated with increased hospital admission and other parameters of asthma-related morbidity. Eggleston et al[20] demonstrated a clear relationship between current exposure to Bla g 1 and sensitization to cockroach allergen in asthmatic children 4 to 9 years old living in inner-city Baltimore. The best correlation between sensitization and allergen concentration was found in the bedroom, even though the highest levels of cockroach allergens were in the kitchen. The proportion of children with a positive skin test response to cockroach allergen increased as the level of exposure to Bla g 1 increased, from 32% among children exposed to 1 to 2 U/g to a plateau at 40% to 45% among those exposed to concentrations higher than 4 U/g. These data have been confirmed in case control studies of US schoolchildren, which showed that the degree of cockroach sensitization, as determined by skin prick test, was associated with increased exposure to the cockroach allergen Bla g 2 (Table I).[13] A 4-fold increase in median Bla g 2 concentration resulted in significant increases in the numbers of children who became sensitized (≥4-mm wheal) or strongly sensitized (≥8-mm wheal). It has been recently shown that exposure to detectable levels of the cockroach allergen Bla g 1, as well as to high levels of mite allergens (>10 μg/g), in house dust was associated with a higher risk of development of positive skin test responses to the respective allergens in 5- to 12-year-old asthmatic children across North America.[21] In addition to allergen exposure, the degree of atopy, as assessed by the total number of positive skin test responses or by total serum IgE levels, was an important contributing factor for sensitization. These studies provided the basis to propose threshold levels of cockroach exposure above which susceptible individuals would be at an increased risk for sensitization or asthma symptoms. These levels have been defined as 2 U/g and 8 U/g of allergen, respectively. However, at present, it is not clear when sensitization to cockroach allergen occurs and where the main exposure takes place.

Emergency department (ED) studies of asthma carried out in Charlottesville, Virginia; Atlanta, Georgia; and Wilmington, Delaware demonstrated that sensitization to cockroach was an important risk factor associated with asthma admissions to the ED for both adults and children.[6-9] In general, asthmatic patients allergic to cockroaches are exposed to high levels of cockroach allergens in their homes. Measurements of Bla g 1 and Bla g 2 in house dust show a modest but significant correlation, and usually both allergens can be detected in cockroach-infested houses (Fig 1). In the United States high levels of Bla g 1 or Bla g 2 in house dust have been associated with urban residence, African American race, and low socioeconomic status.[8,22]

It is clear that there is a dose-response relationship between exposure to dust mite allergens and sensitization, and this also appears true for cockroach allergen (Table I).[23] Results of prospective studies have shown that early exposure to mite allergens is a significant pre-

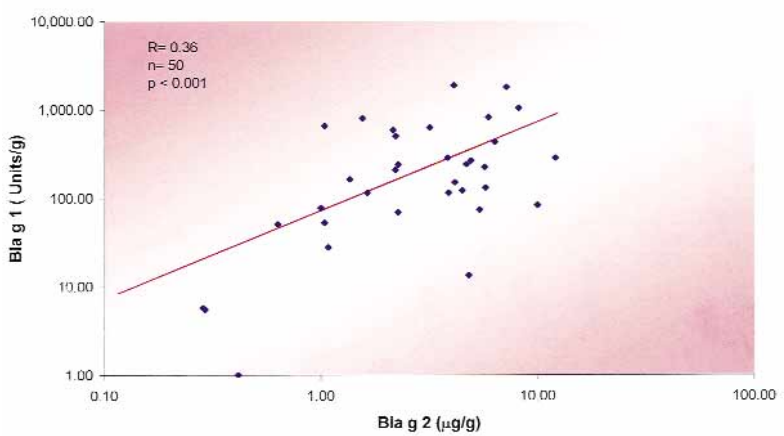

FIG 1. Correlation between ELISA measurements of Bla g 2 and Bla g 1 in dust samples from 50 cockroach-infested homes in the United States. Most samples contained readily detectable levels of either allergen, although the overall correlation between allergen levels is not strong. Little is known of the factors that control the production of these allergens in cockroaches.

TABLE I. Cockroach sensitization, asthma, and domestic allergen exposure*

	Atopic children sensitized to cockroach allergen, % (n)		Children with asthma, % (n)		
Bla g 2 (μg/g), median (range)	Wheal diameter >4 mm	Wheal diameter >8 mm	Atopic	Cockroach sensitive	Nonatopic
<0.08	20 (29/145)	3 (4/145)	29 (41/145)	34 (10/29)	6 (7/119)
0.33 (>0.08-15)	72 (13/18)	28 (5/18)	39 (7/18)	38 (7/18)	25 (5/20)
	$P < .005$	$P < .001$	$P = .5$	$P = 1.0$	$P = .015$

*Multicenter case control studies in 3 US schools in Los Alamos, New Mexico, and central Virginia.[13]

dictor of sensitization and asthma.[24] However, the early immunologic events leading to sensitization have not been clearly defined. Some researchers have reported that sensitization to mites may occur as early as during gestation, as judged by the development of proliferative T-cell responses to mite allergens in cord blood samples.[25-27] However, it is unlikely that enough mite (or cockroach) allergen could cross the placenta and trigger a T_H2-specific response in the fetus, even among mothers exposed to high levels of allergen during pregnancy.[23]

An ongoing large epidemiologic longitudinal study of children born to parents with asthma, allergies, or both living in metropolitan Boston has revealed interesting aspects of the relationship of cockroach exposure early in life and the subsequent development of wheezing and asthma. Exposure to cockroach allergens in the first 3 months of life correlated with the development of repeated wheezing in the first year. The correlation between levels of Bla g 1 or Bla g 2 greater than 0.05 U/g of dust in the family room and repeated wheezing continued to be significant after adjustments for socioeconomic factors, such as income and race.[28] These cockroach allergen levels are very low when compared with allergen levels reported in previous ED studies and in the National Cooperative Inner City Asthma Study (Fig 2).[8,9,12,19,28-30] Studies of siblings of the index children participating in the Boston cohort showed that exposure to cockroach allergens early in life was also associated with doctor-diagnosed asthma and recurrent wheezing over a 22-month follow-up period.[29] Strikingly, children exposed to greater than 2 U/g Bla g 1 or Bla g 2 in the family room or kitchen had a relative risk for incident asthma of 35% and were at risk for recurrent asthmatic wheezing. In the Boston study, which did not include analysis of bedding or bedroom floor dust samples, neither dust mite nor cat allergen levels were predictive of incident asthma or recurrent asthmatic wheezing.[29] Consistent IgE antibody responses to inhalant allergens are usually not detected before the age of 2 years, even though asthma symptoms first occur during infancy and early childhood in most asthmatic children.[23] It has been reported that children exposed to Bla g 1 or Bla g 2 in the first 3 months of life have lymphocyte proliferative responses to Bla g 2 by the age of 2 years.[30] Exposure to greater than 2 U/g Bla g 1 or Bla g 2 in the kitchen predicted increased lymphocyte proliferation. A similar, although less marked, correlation has been observed for exposure to high levels of Der f 1 (≥10 μg/g) in the family room and lymphocyte proliferative responses to this mite allergen, whereas no correlation was found for levels of Fel d 1 and the respective Fel d 1–specific response.[30]

The fact that early exposure to cockroaches, but not to mites or cats, was associated with recurrent wheezing and asthma independent of specific IgE antibody responses raises several issues regarding the role of cockroach allergens in sensitization. It could be speculated that cockroach allergens promote sensitization through direct proinflammatory effects on the lungs. Many aller-

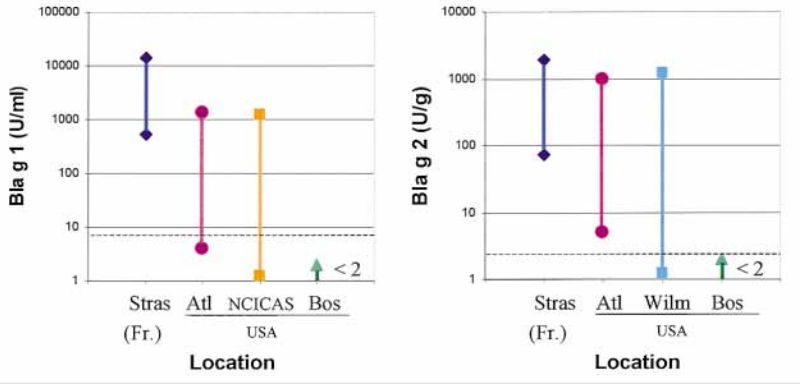

FIG 2. Environmental exposure to cockroach allergens. The range of allergen levels reported in house dust samples as part of studies in several locations is compared: Strasbourg, France *(Stras)*[19]; Atlanta, Georgia *(Atl)*[9]; National Cooperative Inner City Asthma Study *(NCICAS)*[12]; Wilmington, Delaware *(Wilm)*[8]; and Boston, Massachusetts *(Bos)*.[28-30] Exposure levels associated with sensitization are indicated by *dashed lines*. Clinically significant levels in the Boston study were reported for Bla g 1 or Bla g 2 of 0.05 2 U/g.

gens have sequence homology with known enzymes or have functional enzymatic activity.[31,32] Several mite allergens are proteolytic enzymes (cysteine and serine proteases, trypsin, and chemotrypsin), and it has been proposed that proteolytic activity directly contributes to the allergenicity of mite proteins, facilitating penetration through mucosal surfaces and induction of IgE antibody response.[31] Der p 1 and other mite allergens cause disruption of intercellular tight junctions, detachment of lung epithelial cells, and release of inflammatory cytokines in vitro.[32] However, there is no evidence for similar enzymatic effects with cockroach allergens. The major cockroach allergen Bla g 2 is a 36-kd protein found in cockroach gut tissues, which shows sequence homology to aspartic proteinases.[33] However, Bla g 2 has critical substitutions in the enzymatic active site and is an inactive proteinase closely related to a group of mammalian pregnancy–associated glycoproteins.[34] Other cockroach allergens do not appear to have proteolytic activity. The group 1 cockroach allergens (Bla g 1 and Per a 1) have an unusual structure, consisting of a series of up to 14 tandem repeats, each approximately 100 amino acid residues in length, and show 30% homology to a mosquito *(Anopheles gambiae)* protein precursor, ANG12, which is secreted only in the female insect after a blood meal (Fig 3).[35-39] Bla g 4 belongs to the lipocalin (or calycin) family of proteins, which are extracellular proteins that bind and transport small hydrophobic molecules.[40,41] Other *B germanica* allergens include Bla g 5, which is a member of the glutathione-S-transferase (GST) family of enzymes.[42] Patients living in Taiwan have IgE antibodies to allergens from *P americana*, including Per a 3, an insect storage protein related to arylphorin.[43] More recently, the structural protein tropomyosin has been identified as a major allergen in *P americana*.[15] Positive skin test responses, serum-specific IgE antibodies, or both to each of the purified cockroach allergens have been reported, indicating that these proteins are able to induce strong IgE antibody responses in susceptible individuals. At present, there is no evidence that cockroach allergens act on the respiratory tract other than by means of IgE-mediated sensitization. In addition, it is not clear whether cockroach allergens are more potent than other allergens in eliciting allergic responses.

COCKROACH-INDUCED ASTHMA: CLINICAL PRESENTATION

Typically, the clinical presentation of asthmatic patients with cockroach allergy is nonspecific.[44] Most commonly, patients have a history of perennial asthma, possibly worse in the winter,[45] without a clear history of onset of symptoms on exposure to cockroaches. Patients usually show sensitivities to multiple indoor and/or outdoor allergens, although some may be exclusively allergic to cockroaches. Diagnosis relies on the physician inquiry about cockroach infestation and on inclusion of cockroach extracts in allergy skin testing or measurements of specific IgE levels in the serum. Domiciliary cockroach species tend to spend most of the time hidden from sight and aggregate around dark and humid places, and therefore cockroach infestation may not be apparent to the patient. Gelber et al[8] showed that 20% of homes with no visible evidence of cockroach infestation had detectable Bla g 2 levels in at least one site of the house. In addition, cockroach aeroallergen particles have similar properties to mite allergens in that they are relatively large (>10 mm in diameter) and only detectable after disturbance.[19,46] In agreement with these observations, patients are usually not aware of being allergic to cockroaches.

Kang et al[45] reported that asthmatic subjects with cockroach allergy had longer durations of asthma and showed a higher proportion of steroid dependency, suggesting more severe disease, compared with patients allergic to ragweed. A trend toward a significant association of high levels of IgE to cockroach allergen (>60 kU/L) and asthma severity has been described in pregnant inner-city women.[47] Although it is possible that cockroach allergens induce particularly severe and chronic asthma, other factors, including exposure to high levels of cockroach aller-

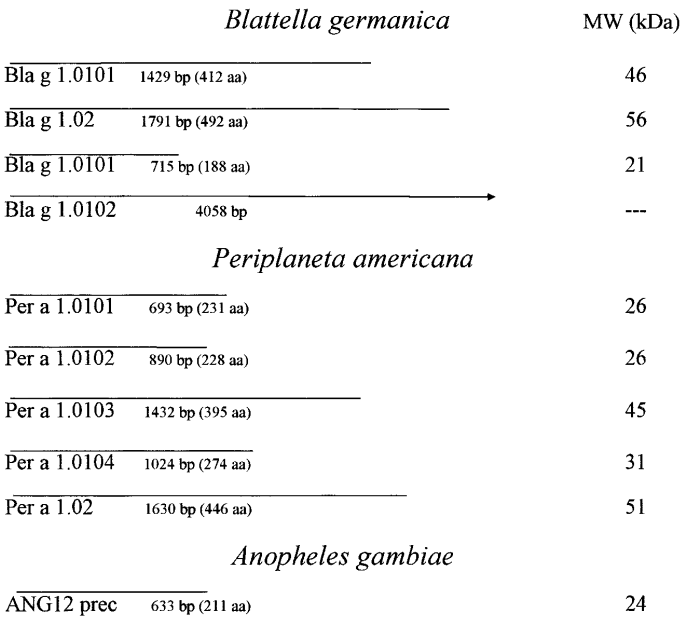

FIG 3. Isoforms and variants of the group 1 cockroach allergens Bla g 1 and Per a 1. These allergens are produced in the gut and have multiple trypsin cleavage sites, which are in part responsible for the multiple molecular forms.[35-36]

gens in the home, poverty, limited access to medical care and medication, overcrowding, and smoking, could play a role in the severity and persistence of asthma among asthmatic subjects with cockroach allergy.

CLINICAL SIGNIFICANCE AND IMMUNE RESPONSE TO COCKROACH ALLERGENS: ANIMAL MODELS AND HUMAN RESPONSES

Bronchial provocation studies revealed that most asthmatic patients with positive skin test responses to cockroach extract have an immediate asthmatic response on inhalation of cockroach extract. Patients also have a late asthmatic response after several hours, with significant peripheral eosinophilia 24 hours after challenge. In contrast, asthmatic subjects with negative cockroach skin test responses fail to react to inhalation of cockroach allergen, indicating that the asthmatic response is specific.[4,5]

Animal models have been developed to investigate the pathogenic role of cockroach allergens in asthma. Guinea pigs sensitized with chronic aerosolized cockroach extract without adjuvant have positive skin test reactions and airway obstruction, along with the release of leukotrienes, in association with production of IgG1a-type reaginic antibody but not IgE.[48] Cockroach inhalation provoked leukocytosis and eosinophilia in the bronchoalveolar lavage fluid only in sensitized animals. In a murine cockroach asthma model, mice were systemically immunized with cockroach extract in incomplete Freund's adjuvant, followed by intranasal administration of cockroach allergen and intratracheal primary and secondary challenges.[49] Primary allergen challenge elicited a peribronchial inflammatory response characterized by eosinophil accumulation and airway hyperreactivity. Repeated allergen exposure increased the extent and rate of eosinophilia, leading to prolonged airway hyperreactivity. Although the responses to inhaled cockroach antigens in these animal models resembled those that have been observed in asthmatic patients with other allergens, the interpretation of the results is limited by the use of heterogenous antigen extracts in the models. Thus the observed effects, however compelling, cannot be attributed to responses induced by specific allergens or correlated with allergens that are known to cause IgE responses in human subjects. It is likely that in human subjects cockroach allergens are potent inducers of inflammation, consisting of mononuclear cells and eosinophils, and of bronchial hyperreactivity as a consequence of chronic inhalation of cockroach allergens present in the indoor environment.

COCKROACH-INDUCED ASTHMA: IMPLICATIONS FOR THE HYGIENE HYPOTHESIS

Several hypotheses have been raised to explain the steady increase in asthma prevalence and morbidity over the past 2 decades in developed countries, including changes in lifestyle, with more time spent indoors and increased exposure to indoor allergens; lack of exercise; increase in obesity; and dietary changes.[50] However, no unified explanation is available. Evidence has suggested that the increase in asthma prevalence has been associat-

ed with improvements in hygiene and health standards and with reduction of family size.[50,51] According to the hygiene hypothesis, a reduction in early exposure to microbes would allow neonatal T_H2 responses developed during gestation as a part of the mechanism for successful pregnancy to become established. These T_H2 responses (presumably directed toward allergens) would replace the T_H1 responses characteristic of nonallergic individuals. Epidemiologic evidence in favor of this simple hypothesis includes a decrease of asthma in children who attended day care or had older siblings, in children of anthroposophic families, and in children raised in rural environments with animals near the house.[51] However, the link between these epidemiologic observations and specific changes in immune responsiveness is tenuous and based largely on speculation. Viral infections and allergen sensitization are clearly important risk factors for the development of asthma and nutrition, and other environmental factors in the first 2 years of life may also play a role.[23] A decrease in parasitic infections, which are potent inducers of IgE synthesis and eosinophilia (T_H2-type responses), has also been suggested to contribute to increases in allergy.[50]

Results of the International Study of Asthma and Allergies in Childhood showed a striking variation in the prevalence of asthma among countries throughout the world. Underdeveloped countries did not have lower prevalences of asthma compared with those in the developed world.[52] In Brazil, for example, the 12-month prevalence of self-reported asthma symptoms among 13- to 14-year-old children ranged from 15% to 25%, which is higher than that of the United States and several European countries. Most children with asthma living in Brazil are allergic to dust mites and cockroaches, as judged by the presence of positive skin test responses and serum-specific IgE antibodies, and are exposed to high levels of mite allergens in their homes.[15] In addition, most children under 2 years of age presenting to an ED with acute wheezing were exposed to levels of cockroach allergen above those proposed as risk factors for sensitization and associated with development of recurrent wheezing.[53] Although the population in Brazil and in other developing countries is distributed throughout a wide range of socioeconomic status, most children live in poor conditions and have frequent viral, bacterial, and parasitic infections. It is unlikely that poor hygiene is protecting these children from having asthma. However, other factors, such as genetic influences, may play a role in this complex interaction of environmental factors and development of asthma.

In the United States the increase in asthma prevalence and mortality has been disproportionately higher among African Americans.[54] In a study of asthmatic children in metropolitan Baltimore, African American race and low socioeconomic status were independently associated with sensitization to cockroach allergen.[22] High levels of cockroach allergens were found in homes of low-income, inner-city families, which were heavily infested with cockroaches. These homes also accumulated large amounts of food wastes and debris in the kitchens and were often unsanitary. In this scenario one would not expect a decreased rate of infections in early childhood, and yet the prevalence of asthma appears to be high. Indeed, it seems naive to assume that the urban poor do not have similar exposure to T_H1-like adjuvants as rural families. The implication of these observations is that the hygiene hypothesis does not adequately explain the rise in asthma prevalence seen in many areas of the world.

CROSS-REACTIVITY AMONG INSECT, MITE, AND FOOD ALLERGENS

There is an increasing body of evidence that cross-reactivity occurs among members of the class Arthropoda, particularly crustaceans, insects, and arachnids, and also with other invertebrates, such as mollusks and nematodes. In vitro IgE cross-reactivity between mites and cockroaches and foods derived from invertebrates, including shrimp, other crustaceans, and snails, has been previously demonstrated by inhibition studies. The common allergen that accounts for most of the allergenic cross-reactivity is thought to be the muscle protein tropomyosin.[55] Tropomyosin belongs to a family of highly conserved proteins with a characteristic coiled-coil structure, which is found both in muscle and nonmuscle cells. Tropomyosin has been identified as a major allergen in shrimp (Pen a 1, Met e 1, and Pen I 1) and also in other crustaceans (crab Cha f 1, lobster Pan s 1, and Hom a 1), mollusks (oyster Cra g 1, gastropod Tur c 1, and Tod p 1), and dust mites (Der p 10 and Der f 10).[55-60]

Recently, we have identified tropomyosin as a major allergen in cockroaches. *P americana* tropomyosin, designated as Per a 7, reacts with approximately 50% of sera from asthmatic children allergic to cockroaches.[15] This allergen shows a high degree of sequence identity to tropomyosins from invertebrates, particularly from mites (80% identity) and shrimp (82% identity). An mAb raised against *Dermatophagoides pteronyssinus* (mAb 1A6) shown previously to bind to shrimp tropomyosin also recognized cockroach tropomyosin on immunofluorescence. The cloning of the American cockroach tropomyosin allergen has also been reported by Asturias et al,[61] and a sequence of a *B germanica* tropomyosin with greater than 98% amino acid sequence identity to *P americana* tropomyosin has been recently reported to GeneBank (Accession Number AF260897). Recombinant Per a 7 expressed in *Pichia pastoris* and natural Per a 7 purified from whole-body *P americana* extracts caused positive skin test responses in allergic patients and no reactivity in control subjects.[62]

Cross-reactivity among tropomyosins reflects shared primary and tertiary structures. Although tropomyosins of different origins have a similar fold, differences in the primary sequence between tropomyosins from invertebrates and vertebrates probably account for the fact that vertebrate tropomyosins are not allergenic. Typically, invertebrate tropomyosins share 70% to 80% amino acid sequence identity and show only 50% to 60% amino acid

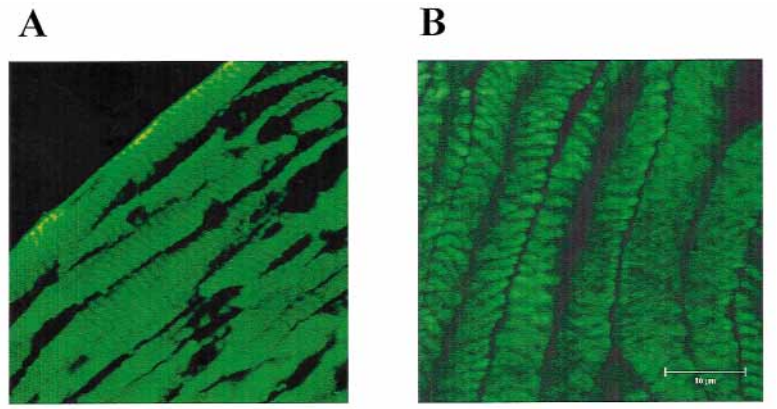

FIG 4. Immunofluorescence analysis of unfixed frozen sections of cockroach and *Ascaris lumbricoides*. **A,** Section of cockroach immunostained with mAb 1A6 and *Dermatophagoides pteronyssinus* tropomyosin. The banding pattern typical of striated skeletal muscle is apparent (magnification, 300×). **B,** Section of *Ascaris lumbricoides* stained with mAb 1A6 showed a similar banding pattern. The muscle fibers are positive for tropomyosin (magnification, 100×). Control sections stained without the primary antibody did not show fluorescence.[31]

sequence identity to vertebrate tropomyosins, including the one of human origin. Subjects with a history of allergy to dietary meats show IgE binding to proteins other than tropomyosin, suggesting that tropomyosin is not an important vertebrate meat allergen. IgE antibodies to shrimp tropomyosin do not cross-react with homologous mammalian tropomyosins from pork, beef, chicken, or mouse. Detailed studies of these nonallergenic counterparts may shed light on the structural features that contribute to the allergenic activity of invertebrate tropomyosins.[55]

It is not clear at present whether allergenic cross-reactivity among mites, cockroaches, and foods are clinically relevant. Symptoms of food allergy after ingestion of invertebrates (shrimp and snails) have been reported in European patients allergic to mites, and many of these patients receive mite immunotherapy. Van Ree et al[63] have shown that the development of IgE to shrimp and snails in 3 of 17 patients receiving mite immunotherapy was accompanied by oral allergy symptoms on ingestion of shrimp in 2 of the patients. These patients had serum IgE to cross-reactive tropomyosin from mites, shrimp, and snails and positive skin prick test responses to shrimp. This study suggested that increased exposure of some patients to mite allergens (through immunotherapy) results in sensitization to cross-reactive seafood tropomyosins.

Several studies have demonstrated cross-reactivity of IgE to insects and parasites, particularly the nematodes *Anisakis simplex* and *Ascaris suis*, which may also be caused by tropomyosin.[55] We have recently investigated the role of *Ascaris lumbricoides* infestation in IgE responses to inhalant allergens in children of low socioeconomic families with asthma, rhinitis, or both living in Brazil. Our results showed that 61% of these children presented serum IgE antibodies to *Ascaris lumbricoides*. The presence of IgE to *Ascaris lumbricoides* associated with IgE to inhalant allergens caused a significant increase in total IgE levels.[64] Monoclonal antibody 1A6 showed strong binding to *Ascaris lumbricoides* striated muscle tissue by means of immunofluorescence (Fig 4). Partial sequencing of a PCR-amplified *Ascaris lumbricoides* cDNA fragment, obtained by using tropomyosin-specific primers, revealed a high degree of sequence identity to invertebrate tropomyosins, including those from *Anisakis simplex*, cockroaches, and mites (unpublished observations). Our results support the hypothesis that tropomyosin is an important protein involved in cross-reactivity among insects, mites, crustaceans, mollusks, and parasites.

Other cockroach allergens have been assigned to families of proteins with homologies at the level of primary structure or with protein-folding similarities. Bla g 5 shows 40% to 50% homology to other insect GSTs and 28% homology to the house dust mite GST allergen (Der p 8).[42] Molecular modeling of the cockroach allergen Bla g 4 revealed that it shares the tertiary structure of other members of the lipocalin family.[40,41] However, at present, is not clear whether these similarities would have any role in cross-reactivity with mites or animal allergens, including those of dog, cow, or horse dander or of cow's milk. In many areas of the world, patients are simultaneously exposed to mites and cockroaches and become sensitized to both allergens. Prospective studies would be necessary to determine the specificity of the IgE response and the routes of initial sensitization in these patients.

ALLERGENIC IMPORTANCE AND POTENTIAL DIAGNOSTIC AND THERAPEUTIC APPLICATIONS OF COCKROACH ALLERGENS

Traditional methods of allergy diagnosis and treatment have relied on the use of heterogeneous allergen extracts. The problems with the use of these extracts include difficulties in assessing their potency and the possibility of anaphylactic reactions occurring during immunotherapy.[32] Most cockroach allergens have been cloned and expressed

as recombinant proteins, and the reactivity of recombinant allergens is comparable with that of their natural counterparts.[65] The advantage of using recombinant allergens for diagnosis is that they can be produced on demand in large quantities with a high degree of purity. Therefore it is necessary to investigate whether cocktails of selected allergens would be suitable for clinical purposes and to determine the appropriate combination and doses of allergens that should be included in these cocktails.

Previous results of skin tests and in vitro serologic assays have established that the prevalence of IgE antibodies to natural Bla g 1 and its homologue Per a 1 ranges from 30% to 50% (that to natural Bla g 2 is 60% to 80%) among patients with positive skin test responses to cockroach allergen. Recombinant cockroach allergens have been produced in *Escherichia coli* (Bla g 4, Bla g 5, Per a 1, and Per a 3) or in the yeast *Pichia pastoris* (Per a 1, Bla g 2, Bla g 4, and Per a 7). Serologic studies suggest that the prevalence of IgE antibodies to rBla g 4 and rBla g 5 is 60% and 70%, respectively, and that a cocktail of Bla g 1, Bla g 2, Bla g 4, and Bla g 5 would diagnose 95% of US cases of cockroach allergy.[65] Current data demonstrate that Per a 1, Per a 3, and Per a 7 are important *P americana* allergens that should be considered for research and clinical use. Further clinical trials of cockroach recombinant allergens will be necessary to establish the role of these allergens in the diagnosis of cockroach allergy and to determine the optimal concentrations to be used for skin testing.

Immunotherapy with cockroach extract has been reported to lead to beneficial changes in immunologic and clinical parameters in one small study carried out in 1988.[66] At present, cockroach immunotherapy is not considered to have proven efficacy. Although the indications for immunotherapy in patients with asthma remain controversial, several well-designed placebo-controlled studies have demonstrated therapeutic benefits of specific immunotherapy in patients with asthma who are allergic to mites, animals, and pollens.[67] Success in immunotherapy with natural allergenic products may be improved with the use of standardized extracts, and the development of standardized cockroach extracts is currently being pursued by the US Food and Drug Administration.

The production of recombinant cockroach allergens is expected to facilitate the development of improved forms of immunotherapy. Cocktails of recombinant allergens could be used in conventional immunotherapy protocols. Overlapping peptides, hypoallergenic variants of allergens generated by site-directed mutagenesis, and the use of T_H1 adjuvants are other novel strategies.[68-73] Plasmid DNA vaccines have been studied in experimental animals, and the results showed that they could effectively prevent the development of IgE antibodies and airway hyperresponsiveness when given to naive animals and reduce airway hyperresponsiveness on subsequent exposure to the allergen.[74] Raz et al[75] and Sato et al[76] characterized the bacterial DNA sequences as CpG motifs located outside the allergen gene regions, which were responsible for an adjuvant effect eliciting IFN-γ and IL-12. Subsequently, it has been shown that CpG can be administered together with a protein antigen to induce an antigen-specific T_H1-type response.[77-79] Immunotherapy with the major ragweed allergen Amb a 1 coupled to CpG sequences is being investigated in a clinical trial in patients allergic to ragweed. It is expected that some of these new strategies could be incorporated in the development of new forms of immunotherapy for cockroach allergy, which would be effective for ongoing disease or perhaps for prophylactic immunization.

ENVIRONMENTAL CONTROL

Treatment of cockroach-induced asthma includes recommendations to reduce exposure to cockroach allergens in the home and work environments. Unfortunately, cockroach allergens persist in the indoor environment, even after the cockroach population has been greatly reduced.[16-18,80] Intervention based on professional extermination with abamectin resulted in a decrease in Bla g 1 levels in the kitchen, although only for a short time. The reduction achieved was to levels still above those considered as clinically significant.[16] More recently, Eggleston et al[17] have studied the effect of professional extermination with 0.05% abamectin and professional house cleaning on cockroach infestation and allergen levels in inner-city homes in Baltimore. Although such measures resulted in a cockroach population decrease and a substantial reduction of allergen levels in some homes, levels at the end of the 8-month study period were still above 20 U of Bla g 1 per gram in most homes. Similarly, Williams et al[18] have also reported successful cockroach extermination with hydramethylnon; however, elevated Bla g 1 and Bla g 2 levels persisted for up to 6 months after treatment. These studies demonstrate that the current pesticides applied by professional pest control technicians are effective. However, sustained removal of cockroach allergens from homes may be difficult to achieve. Cockroach extermination needs to be done in all rooms and should be coupled with thorough cleaning measures, addressing reservoirs of allergen in carpets, rugs, and other sites, and efforts to prevent reinfestations to achieve effective control of allergen exposure in the household.

CONCLUSIONS

Recent studies have shown that early exposure to cockroach allergens in children born to parents with asthma, allergies, or both may contribute to the development of recurrent wheezing and doctor-diagnosed asthma in early childhood. Data on sensitization to cockroach allergen in these patients are lacking. However, it has been shown that exposure to cockroach allergen in early life is associated with increased lymphocyte proliferative responses to cockroach allergen by the age of 2 years. These effects have occurred at exposure levels well below the currently proposed thresholds, raising the possibility that in susceptible children lower levels may cause symptoms and contribute to disease. On the basis

of these observations, recommendations aimed at reducing exposure to cockroach allergens could be helpful for susceptible children. Results of recent studies showed that significant reduction in cockroach allergen levels in homes is difficult to achieve, despite successful cockroach extermination. In addition, sensitization to cross-reactive allergens present in mites, foods, and parasites, particularly to tropomyosins, may play a role in disease. Much is known about the structure of cockroach allergens, and several of these allergens are currently available as biologically active recombinant proteins. Progress on the molecular biology cloning of cockroach allergens may lead to the development of novel forms of immunotherapy, which might benefit asthmatic patients with cockroach allergy and could potentially be used as vaccines to prevent disease in the future. However, the fact remains that cockroach-induced asthma is essentially a disease caused by poverty, which could be eliminated through improvements in housing conditions and patient education. Action in these areas, as well as improved asthma management and environmental interventions, will be critical in reducing the prevalence of asthma in cockroach-infested housing.

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