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Correlates of Sensitization to *Blomia tropicalis* and *Dermatophagoides pteronyssinus* in Asthma in Barbados

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Key Words
Asthma · IgE · Dust mites · Barbados

Abstract
Background: Sensitivity to the mite *Blomia tropicalis* is related to asthma in tropical climates, but correlates of sensitivity to *B. tropicalis* and its relationship to *Dermatophagoides pteronyssinus* sensitivity have not been widely examined in families with asthma. The main objective of this study was to determine prevalence and correlates of sensitivity to these mites in families with asthma and characteristics of persons sensitized to both. ***Methods:*** Antibodies to major antigens (Blo t 5 and Der p 1) of these mites were measured by immunochemiluminescent assay in 481 members of 29 families from Barbados ascertained through two asthmatic siblings. ***Results:*** Blo t 5 sensitivity was present in 261 subjects (46%) and was associated with younger age, higher total serum IgE level, and more than a three-fold increased prevalence of asthma (42 vs. 13%). Der p 1 sensitivity was less common (27%) and showed similar associations with age, IgE, and asthma. Of the 261 subjects sensitized to Blo t 5, 116 were also sensitized to Der p 1; they were younger, had higher total and Blo t 5 specific IgE levels, and had more than twice the asthma prevalence as those sensitized to Blo t 5 alone (59 vs. 29%). Der p 1 sensitivity without Blo t 5 sensitivity was uncommon; 90% of those sensitized to Der p 1 were also sensitized to Blo t 5. Geometric mean total IgE levels were lowest in the 207 participants without any mite sensitization (102 U/ml), intermediate in 158 sensitized to *either* Blo t 5 *or* Der p 1 (609 U/ml), and highest in 116 sensitized to both (1,869 U/ml). ***Conclusions:*** Blo t 5 is the predominant sensitizing mite allergen in these Barbadian families with correlates similar to Der p 1. Concomitant sensitization to Der p 1 appears to identify a more reactive subgroup of individuals at a higher risk of asthma.

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Introduction

Asthma has been linked etiologically to hypersensitivity to a variety of common indoor allergens, including dust and storage mites [1, 2]. Sensitivity to dust mites of the

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genus *Dermatophagoides* has been widely studied in relation to asthma in temperate [3, 4] and tropical [5, 6] climates. Extensive birth cohort studies have demonstrated that mite sensitization itself is a strong predictor of subsequent asthma, wheezing, and bronchial responsiveness, while indoor exposures to allergens are not directly related [18]. Sensitivity to the storage mite *Blomia tropicalis,* which frequently coexists with *Dermatophagoides* species [8], is related to asthma in tropical climates and has been suggested to be the primary sensitizing agent among asthmatics in Brazil, Venezuela, and Colombia [8, 9]. Although sensitivity to both mites is frequent [8, 10], the correlates of dual sensitivity and its relationship to asthma and total IgE levels have not been widely explored in areas where both mites are common.

Most studies of sensitivity to *B. tropicalis* and *Dermatophagoides* spp. in tropical areas have demonstrated prevalences exceeding 80–90% in subjects with asthma and/or atopy, with few subjects sensitized to one but not the other [10–12]. One report from a subtropical region (Florida) demonstrated lower prevalence of sensitization and comparable proportions of subjects sensitized to both *B. tropicalis* and *Dermatophagoides pteronyssinus* as to *D. pteronyssinus* alone (35 vs. 27%) [13]. Information is limited about the correlates of mite sensitization (particularly for *B. tropicalis*), such as environmental factors, symptoms, and IgE levels. Characteristics of persons sensitized to both mites and comparison to those sensitized to only one have not been described.

Barbados is a tropical country with a high prevalence of both *B. tropicalis* and *D. pteronyssinus* infestation in homes [8–10]. Data from the Hopkins-Barbados Study on the Genetics of Asthma and Allergy were analyzed to: (1) determine the prevalence of IgE specific to these two indigenous dust mites in 481 members of 29 Barbadian families ascertained through two asthmatic siblings; (2) describe distributions of IgE levels to the major antigens of these mites (Blo t 5 and Der p 1) and identify their correlates, and (3) identify differences in patterns of sensitization to Blo t 5 and Der p 1 and determine potential unique characteristics of persons sensitized to both.

Patients and Methods

Data Collection

Asthmatic probands were identified from patients with a documented history of asthma seen in public and private clinics and in the Accident and Emergency Department of Queen Elizabeth Hospital in Barbados [14]. Patients with a positive family history of asthma or one or more asthmatic siblings were referred for study by physicians cooperating with on-site study investigators at the University of the West Indies. After giving informed consent, available first-degree relatives and other family members were recruited into the study between April 1993 and December 1996. Data were available on 481 subjects in 29 extended families, including 49 asthmatic probands, 108 asthmatic relatives, and 370 nonasthmatic relatives. A total of 119 subjects was children under age 12; all subjects but 1 were Afro-Caribbean (race self-reported as 'Black').

Family structure and family history information were obtained by self-report from a responsible family member. Self-reports of asthma-related symptoms as well as other allergic conditions and indoor furnishings and plumbing were obtained by a validated, interviewer-administered questionnaire [15, 16] for adults or through proxy report by a responsible adult for children under age 12. Asthma was defined as a reported history of asthma by standardized questionnaire *plus* confirmation by interview of the patient by a coinvestigator who was one of the primary diagnosticians of asthma in Barbados.

Laboratory Procedures

Phlebotomy was performed under sterile conditions in a nonfasting state. Blood was drawn into Vacutainer tubes and centrifuged to separate plasma and serum. Samples were shipped on ice to the Johns Hopkins Asthma and Allergy Center for IgE measurements and extraction of DNA. Total and allergen-specific serum IgE levels were measured in duplicate using the immunochemiluminometric Magic Lite assay (Magic Lite Total IgE Extended Range; Ciba-Corning, Medfield, Mass., USA), expressed in units per milliliter. The immunochemiluminometric assay utilizes antigen or antibody covalently bound to a luxogenic, or light-producing, substance, such as isoluminol or acridinium ester [17]. Acridinium ester is the chemiluminescent agent used in the Magic Lite immunoassays which utilize specific allergen (such as Der p 1, Blo t M, or a complex of multiple antigens) covalently bound to paramagnetic particles and reacting with allergen-specific IgE in a patient's sample [18]. Solid-phase *D. pteronyssinus* allergens used in the Magic Lite SQ Specific IgE Immunoassay System were derived from highly standardized allergen extracts of mite fecal pellets. The quality of these extracts was assured by quantitative immunoelectrophoresis and cross-radioimmunoelectrophoresis [19]. *B. tropicalis* antigen was prepared and quality assured in a similar manner and provided by Allergologisk Laboratorium (Hørsholm, Denmark). Antibody was acridinium ester labeled mouse antihuman IgE antibody in phosphate-buffered saline with protein stabilizers and sodium azide preservative. High and low calibrators and controls were provided by the manufacturer and run in duplicate with each assay. Units of measure were 'relative light units', equivalent to one photon count on a reference instrument and were converted to standardized units of specific IgE by plotting a standard ten-step serial dilution curve ranging from undiluted to 1:512 [18]. The assay had a minimum detectable concentration of 1.43 standardized units/ml and correlated with radioimmunoassay methods with a correlation coefficient of 0.82 and a slope and intercept of 5.33 and 0.17 U/ml, respectively [18]. Intra-assay coefficients of variation (CV) ranged from 6.2 to 8.9% across concentrations from <4 to 800 U/ml, with CVs being higher at the lower end of concentration. Total CVs ranged from 8.0 to 16.0%, again being highest at low concentrations [18]. Despite the known 43% sequence homology between Blo t 5 and Der p 5 (a minor antigen of *D. pteronyssinus*), cross-reactivity of Blo t 5 and Der p 1 is minimal due to the lack of similarity between the major antigens of the two mites [20].

Table 1. Frequency of sensitization to Blo t 5 and Der p 1 ($\chi_1^2 = 90.3$, $p \leq 0.0001$)

Blo t 5	Der p 1					
	sensitized		not sensitized		total	
	n	%	n	%	n	%
Sensitized	116	44.4	145	55.6	261	54.3
Not sensitized	13	5.9	207	94.1	220	45.7
Total	129	26.8	352	73.2	481	100

Statistics

Distributions of IgE levels were investigated as both categorical and continuous variables. Sensitization to Blo t 5 or Der p 1 was defined as the presence of any detectable allergen-specific IgE. Levels of IgE were $\log_{10}$ transformed for examination as continuous variables. Dual sensitization, defined as four categories (Blo t 5 –, Der p 1 –; Blo t 5 +, Der p 1 –; Blo t 5 –, Der p 1 +, and Blo t 5 +, Der p 1 +), was used in analysis of variance for continuous variables and chi-square analysis for categorical factors. Associations of log-transformed IgE levels with covariates such as age, sex, symptoms, and indoor exposures were assessed using t tests for discrete variables, analysis of variance for categorical variables, and linear regression and correlation for continuous variables. Indoor exposures such as number of pieces of upholstered furniture were dichotomized above and below the 75th percentile level for the sample as a whole. Data on dates of blood collection were divided by seasons into 'dry' (December through April), 'summer' (May through July), and 'rainy' (August through November). Due to limited sample size, analyses used all family members without adjusting for familial relationships. Given the larger number of comparisons performed, associations were considered significant at $p \leq 0.01$ and marginally significant at $p \leq 0.05$.

Results

Prevalence of Sensitization and Distribution of Mite-Specific IgE Levels

Twice as many participants were sensitized to Blo t 5 as to Der p 1 (table 1). Although nearly half of the 261 participants sensitized to Blo t 5 were also sensitized to Der p 1, Der p 1 sensitivity in the absence of Blo t 5 sensitivity was uncommon; only 6% of those *not* sensitized to Blo t 5 were sensitized to Der p 1. In contrast, 90% of the 129 participants sensitized to Der p 1 were also sensitized to Blo t 5. The frequency of Blo t 5 sensitization in the 139 subjects with asthma was 79%, while the frequency of Der p 1 sensitization was 50%. Forty-nine percent of asthmatics were sensitized to both antigens, 30% to Blo t 5 alone, and 1.5% to Der p 1 alone ($\chi_2^2 = 27.8$, $p \leq 0.001$). The geometric mean level of Blo t 5 IgE was 8.3 (range 0–3,654) U/ml and that of Der p 1 IgE 0.33 (range 0–120) U/ml.

Correlates of Sensitization as Discrete Variable

Asthma was least common in the 207 persons sensitized to neither Blo t 5 nor Der p 1, more common in the 145 sensitized to Blo t 5 alone, and most common in the 116 sensitized to both Blo t 5 and Der p 1 (table 2). Sensitization to Der p 1 alone was so infrequent (n = 13) that reliable conclusions about associations cannot be drawn; the values are shown in table 2 for completeness only. All results were essentially the same whether these 13 persons were combined with the 145 sensitized only to Blo t 5 or excluded altogether.

Sensitization to Blo t 5 alone, and particularly to Blo t 5 and Der p 1 concomitantly, was also associated with younger age and higher total IgE levels. The prevalence of symptoms followed a similar pattern, being more frequent in those sensitized to Blo t 5 alone and particularly frequent in those sensitized to both Blo t 5 and Der p 1. These differences were significant for all symptoms and conditions except eczema. Indoor environmental factors were not associated with sensitization. Geometric mean total IgE levels were lowest in those sensitized to neither Blo t 5 nor Der p 1, intermediate in those sensitized to Blo t 5 alone, and highest in those sensitized to both (fig. 1).

Correlates of Mite-Specific IgE Levels

Examination of Blo t 5 and Der p 1 IgE levels as continuous variables also showed associations with asthma and revealed a few additional marginally significant relationships (data not shown). Age-adjusted geometric mean IgE levels were five- to tenfold higher in participants with asthma than in those without. Current cigarette smoking was associated with lower Blo t 5 IgE levels, while indoor toilet and shower, concrete housing, and blood collection during dry and summer seasons were associated with higher levels. The only association observed for age-adjusted Der p 1 IgE levels was with season of blood collection; the levels were highest for collections during the summer. Blo t 5 and Der p 1 IgE levels were negatively correlated with age after childhood, particularly in men

Table 2. Correlates of sensitization to Blo t 5, Der p 1, or both in 481 subjects, tested as four unordered categories

Parameter	n	Blo t 5/Der p 1 sensitization absent (n = 207)	Blo t 5 sensitization present/Der p 1 sensitization absent (n = 145)	Blo t 5 sensitization absent/Der p 1 sensitization present (n = 13)	Blo t 5/Der p 1 sensitization present (n = 116)	p
Asthma	481	27 (13)	42 (29)	2 (15)	68 (59)	0.001
Male sex	481	97 (47)	63 (43)	6 (46)	58 (50)	0.8
Age, years	479	30.7	27.5	32.3	21.7	0.0001
IgE, U/ml						
Total	481	102	627	440	1,868	0.0001
Blo t 5	481	–	23	–	127	0.0001
Der p 1	481	–	–	0.34	4.25	0.0001
Wheeze	435	27 (15)	38 (29)	1 (7.7)	52 (48)	0.001
Cough	436	67 (37)	60 (45)	2 (15)	66 (61)	0.001
Chest tightness	435	40 (22)	40 (31)	3 (23)	64 (59)	0.001
Shortness of breath	433	54 (30)	62 (47)	0 (0)	69 (64)	0.001
Allergic rhinitis	435	71 (39)	77 (58)	6 (46)	79 (73)	0.001
Eczema	432	11 (6.1)	9 (6.9)	0 (0)	11 (10)	0.5
Ever smoker	432	24 (13)	10 (7.6)	2 (17)	10 (9.4)	0.4
Current smoker	432	11 (6.0)	3 (2.3)	0 (0)	3 (2.8)	0.3
Running water	400	141 (83)	106 (87)	8 (73)	84 (87)	0.5
Indoor toilet	400	120 (71)	84 (69)	7 (64)	76 (78)	0.4
Indoor shower	400	124 (73)	86 (70)	8 (73)	78 (80)	0.4
Upholstered furniture (≥ 5)	388	32 (20)	34 (28)	2 (20)	32 (32)	0.16
Housing	428	–	–	–	–	0.3
Wood	–	54 (30)	33 (25)	3 (23)	21 (20)	–
Wood/concrete	–	87 (48)	73 (56)	5 (38)	62 (60)	–
All concrete	–	39 (22)	25 (19)	5 (38)	21 (20)	–
Season of collection	480	–	–	–	–	0.2
Dry	–	90 (44)	67 (46)	7 (54)	68 (59)	–
Summer	–	8 (3.9)	8 (5.5)	0 (0)	5 (4.3)	–
Rainy	–	108 (52)	70 (48)	6 (46)	43 (37)	–

Percentages indicated in parentheses.

(fig. 2). Blo t 5 and Der p 1 IgE levels were also correlated with each other (age-adjusted correlation coefficient = 0.51) and with total IgE (age-adjusted correlations = 0.69 and 0.47, respectively).

Discussion

Blo t 5 was the predominant sensitizing mite allergen in these Barbadian families ascertained through asthma, with correlates similar to those of sensitization to Der p 1. The prevalence of sensitization to Blo t 5 and Der p 1 in this sample was similar to that reported by Caraballo et al. [5] in asthmatics in Cartagena, Colombia (65 and 55%, respectively), as assessed by specific IgE determination. Sensitization in the current study was less frequent, however, than in previous reports, based on skin testing, from other tropical areas. Chew et al. [12], for example, reported positive skin prick testing for *B. tropicalis* and *D. pteronyssinus* in 93 and 96%, respectively, of 289 patients with asthma or allergic rhinitis in Singapore. Similar estimates were reported from skin test results of 203 Singaporeans of unspecified asthma and allergy status studied by Kuo et al. [10]. The prevalence of *B. tropicalis* and *D. pteronyssinus* sensitivity was somewhat lower in 60 Taiwanese in the same study (73 and 88%, respectively) and was lowest (38 and 62%, respectively) among 167 patients with asthma or rhinitis in Tampa, Florida [13]. Some of these differences may be due to variation in assessment of sensitization, as skin prick testing uses crude extracts, often of whole mites, rather than specific purified antigens such as Der p 1 and Blo t 5, as well as different meth-

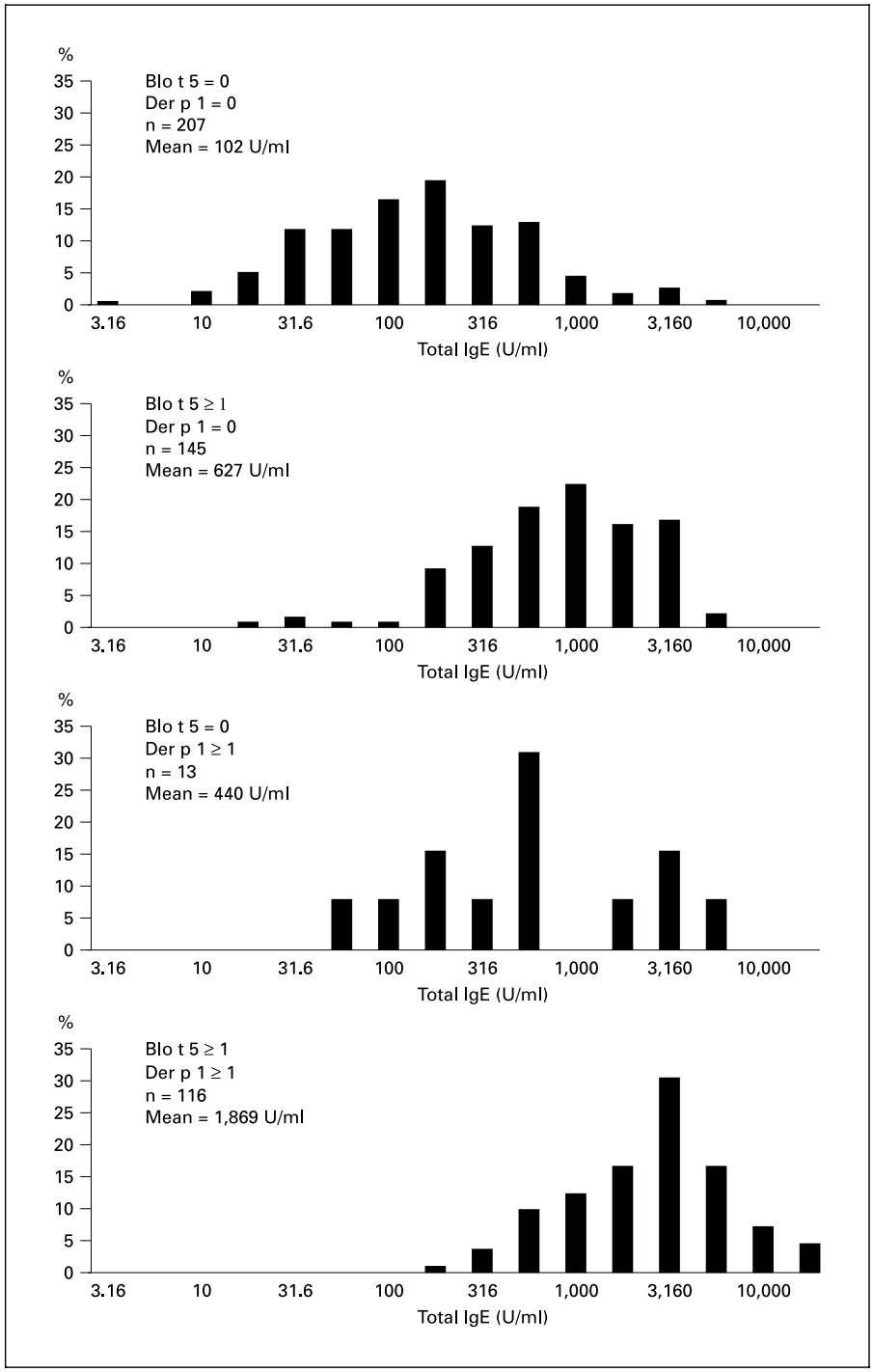

Fig. 1. Total IgE levels by Blo t 5 and Der p 1 sensitization status. Values shown on the x-axis are upper inclusive bounds.

ods to assess sensitization [12]. Observed differences by region may also be due to variation in levels of domestic mite infestation which was not reported for all sites and was not measured in the current study, but has been shown to vary widely [10]. The Barbados data appear unique, however, in the predominance of Blo t 5 as the sensitizing antigen.

Association of allergen-specific IgE levels with asthma, younger age, symptom frequency, and total IgE levels have been reported previously [21, 22]. In contrast to

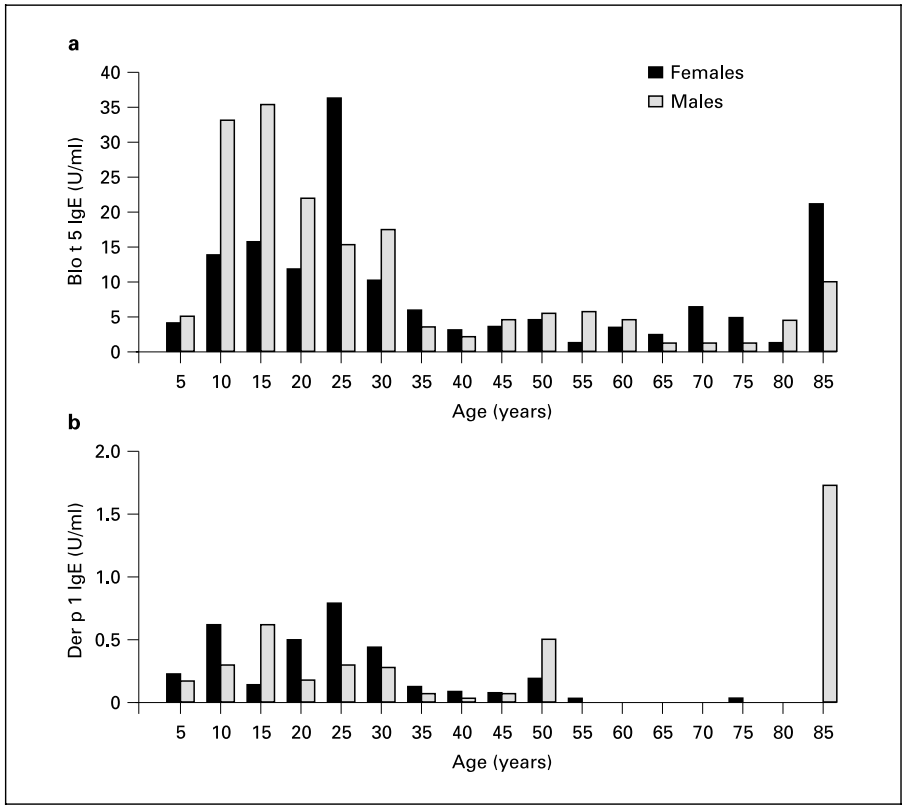

Fig. 2. Geometric mean Blo t 5 (**a**) and Der p 1 (**b**) IgE levels by age and sex. Values shown on the x-axis are upper inclusive bounds. The age strata above 65 years in women and above 55 years in men contain fewer than 5 subjects.

prior studies showing higher IgE levels in smokers [23–25], the current study demonstrated substantially *lower* Blo t 5 IgE levels among current smokers; age-adjusted geometric mean levels were less than 20% of those in current nonsmokers. This might be explained by a 'healthy smoker' effect, consistent with self-selection to lower levels of hazardous exposures in the presence of disease [23, 24], or by chance selection of an atypical subgroup (sampling error).

Dual sensitization in these Barbadian families was less common, even among asthmatics, as compared with the 80–90% rates of dual sensitization reported from other tropical samples [10, 20]. This Barbados sample also demonstrated a distinct imbalance in sensitization between mite species. Prior estimates of dual sensitization, albeit in samples with high rates of sensitization to both mites, have demonstrated nearly as frequent sensitization to Der p 1 in those sensitized to Blo t 5 as the reverse [10–12, 20]. Characteristics of persons sensitized to one or the other of the species, or to both, have not been previously reported, and one might almost infer that lack of sensitization to one mite in the presence of sensitization to the other is almost a chance, and certainly a rare, event.

In contrast, in Barbados, sensitization to Der p 1 was nearly always accompanied by sensitization to Blo t 5, but the converse was far from true. Only the Tampa group [13] demonstrated such an imbalance between mite species, and there the prevalence was reversed, with Der p 1 sensitization being more frequent. Were sensitizations to both Blo t 5 and Der p 1 simply reflections of a hypersensitive immune system reacting indiscriminately to all antigens in its midst, one would not expect dual sensitization to occur in subjects sensitized to Blo t 5 any more frequently than in those sensitized to Der p 1. This was clearly not the case in these Barbadian families, as sensitization to Der p 1 was nearly always accompanied by Blo t 5 sensitization. This suggests that Blo t 5 sensitization predisposes to Der p 1 sensitization, perhaps due to earlier or stronger exposure, or perhaps through some particular stimulation of the immune reaction, in ways that Der p 1 sensitization does not. Another possibility is that Der p 1 sensitization occurs only in a more reactive subgroup of Blo t 5 sensitized individuals which is supported by the higher total IgE levels and the nearly twofold higher asthma prevalence in those sensitized to both antigens as compared with individuals sensitized to Blo t 5 only.

Debate continues [7] as to whether mite sensitization is the initial causative event in asthma etiology or one of several causative factors or simply a marker of increased reactivity also manifest as asthma but caused by some other factor or factors. We believe these data support the latter hypothesis – of sensitization, particularly dual sensitization, as a marker of increased reactivity. Our understanding of the factors related to sensitization itself, however, remains imperfect. Further characterization of factors related to dual mite sensitization in areas where both are common might shed light on the specific mechanisms for these differences.

The use of families in the current study likely introduced some biases by including persons at higher risk of atopic illnesses among the 'nonasthmatics' in this sample, since family members of asthmatics are known to have higher IgE levels and greater atopic tendencies than family members of controls [26, 27]. Inclusion of family as a class variable in analysis of variance models did not alter these results appreciably, suggesting that familial aggregation of environmental and genetic factors cannot explain the observed associations between IgE levels and other correlates, but this possibility should be considered carefully when comparing these results to those from samples of unrelated persons. Conversely, the study of family members might increase the likelihood of identifying associations having a familial basis and thus more likely to be genetic. Because family members share environmental as well as genetic factors, genetic contributions to dual sensitization might best be explored in the presence of extensive environmental measures and assessment of cohabitation. Such measures were not available in the current study, though recent studies have demonstrated mite sensitization as measured by serum IgE levels to be independently predictive of the development of asthma in childhood, while indoor allergen exposures are not [7].

In summary, Blo t 5 is the predominant sensitizing mite allergen in these Barbadian families with asthma, with correlates similar to those of sensitization to Der p 1. Dual sensitization in this population sample appears to identify a higher risk and more reactive subgroup of interest for future studies of asthma and allergic sensitization.

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