

Allergen sensitization of asthmatic and nonasthmatic schoolchildren in Costa Rica

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The prevalence of asthma among schoolchildren in Costa Rica is very high – at the level of 20–30% – and the reason is still unknown. A group of children from our previous epidemiologic study was randomly selected in order to establish the relation between asthma symptoms and allergy sensitization to common allergens. Serum samples from children with and without asthma were analyzed for the presence of IgE antibodies to 36 different allergens, for the presence of IgE antibodies to a pool of 10 common allergens, and for total serum IgE. The most prevalent IgE antibodies were those to mite, cockroach, dog, and house-dust allergens with MAST pipettes for the serologic measurements. Positive reactions to house dust, mite, cat, and the two molds (*Alternaria* and *Cladosporium*), and food allergens such as egg white, peanut, and shellfish were significantly more prevalent among the asthmatics than the nonasthmatics. Sensitization was equally prevalent at different ages, but the house-dust, mite, cat, dog, cockroach, *Alternaria*, and egg-white allergens had sensitized boys more often than girls ($P < 0.01$). The result of the analysis of IgE antibodies to a pool of 10 common allergens by Phadiatop supported the MAST pipette results, showing allergen sensitization in 57.7% of the asthmatic children and 42.3% in the nonasthmatic group. The concentration of IgE was significantly higher among the asthmatic children (372.2 kU/l) than among the nonasthmatic children (249.1 kU/l) ($P < 0.00001$). Parasitic infestations were not examined in this study, but in most of Costa Rica these have largely been eliminated and could not explain the high total IgE levels. Our data indicate that the very high prevalence of bronchial asthma in Costa Rican schoolchildren can be related to sensitization, especially to airborne indoor allergens such as those of mites, cockroaches, and dogs.

An increasing prevalence of childhood asthma is reported from various parts of the world (1–5). Yet the prevalence has rarely reached a level comparable to what was recently found in Costa Rica (6). In a questionnaire-based study, we found bronchial asthma to be present in 23% of schoolchildren. The diagnosis of asthma in the Costa Rican study was based on the response to questions also used in several other studies (4, 6, 7).

The distribution of this questionnaire, the sample selection, and the matching were statistically and epidemiologically designed to give the data a nationwide relevance, as well as indications of geographic, climatic, and socioeconomic influences upon the asthma prevalence. According to many reports (7–13), childhood asthma mainly develops as a typical atopic disease from sensitization to

airborne allergens. In this study, we applied *in vitro* diagnostic methods on a random sample of sera from schoolchildren who participated in the large epidemiologic study to determine the prevalence and specificity of the IgE antibodies in these children. The study offered an opportunity to compare the utility of MAST pipettes and Phadiatop screening disks in a tropical country where diagnostic methods for detecting asthma and allergy are not easily available outside the National Children's Hospital.

Material and methods

From a total of 4026 educational centers, kindergartens, and primary and secondary schools registered in Costa Rica, 98 were randomly selected by

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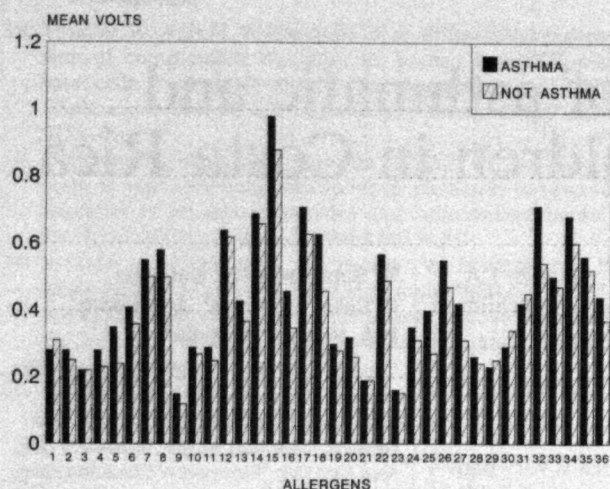


Fig. 1. Mean voltage caused by IgE binding to MAST panel allergens by sera from 171 children with and without asthma. Allergens included in MAST allergy test were as follows. Tree pollen: 1) box elder, 2) cottonwood/willow/aspen mix, 3) elm mix, 4) *Melaleuca*, 5) oak mix; grass pollen: 6) grass mix, 7) Bahia, 8) Bermuda; weed pollen: 9) chenopod mix II, 10) pigweed mix, 11) ragweed mix I, 12) *Rumex* mix, 13) sage mix; dust/mites/insects/epithelials: 14) house dust, 15) mite (*D. farinae*), 16) cat, 17) dog, 18) cockroach mix; molds: 19) *Alternaria*, 20) *Candida*, 21) *Cladosporium*; food: 22) almond, 23) cereal mix, 24) corn, 25) egg white, 26) garlic, 27) milk, 28) onion mix, 29) orange, 30) peanut, 31) potato, 32) shellfish, 33) soybean, 34) vegetable mix, 35) wheat, 36) yeast.

several variables such as urban and rural setting, rain precipitation, temperature, altitude, humidity, and the size of the center. The children from these 98 schools were classified as asthmatics or non-asthmatics by diagnosis by a doctor in combination with the presence of four kinds of respiratory symptoms (cough and wheeze after upper respiratory tract infections or after exercise, as well as frequent daily and/or nightly cough), as reported by the parents in the questionnaire of our previous study (6).

By these criteria, 23% of the children were classified as asthmatics. From subsamples of these groups (asthmatics and nonasthmatics) randomly selected and stratified for geographic location and climate differences, a group of children were chosen to participate in this study.

Other manifestations of atopic disease, such as rhinitis, eczema, dermatitis, and allergic conjunctivitis, were found in 59% of the asthma group and in 39% of the nonasthmatic group.

Serum samples from the asthma and nonasthma groups were selected at random and subjected to two different types of analyses for detection of allergen-specific IgE antibodies.

A total of 171 children were analyzed by the MAST pipette test, 68 asthmatics and 103 non-asthmatics. The Phadiatop analysis was performed in another 347 children, including 175 asthmatics

and 172 nonasthmatics. In only 50 sera, both of these two test methods were used. Total serum IgE was measured in 162 asthmatics and 158 nonasthmatics.

MAST pipette analysis

The MAST pipette (Southern combination panel, IgE, P/N 81085, MAST Immunosystem, Mountain View, CA, USA) included a panel of 36 allergens, 21 airborne and 15 food allergens bound to transversally arranged cellulose threads. Positive and negative control threads were included in each pipette. They were incubated, washed, and reacted according to the manufacturer's recommendation. IgE binding to allergens was determined after photometric recording of the chemiluminescence traces from a substrate activated by enzyme-labeled anti-IgE on a polaroid film.

We used two cutoff levels for defining a positive reaction:

- 1) all reactions giving a luminescence intensity of at least 0.18 V by the MAST luminometer
- 2) reactions giving a luminescence intensity of 0.67 V or more.

Thus, all results were evaluated as positive or negative according to one sensitive and one less sensitive classification. By the first classification, we included as positives both strongly, moderately, and weakly positive reactions. By the second one, only strongly and moderately positive reactions were evaluated as positives. The MAST pipette data were also expressed as the mean V value for each allergen in the asthmatic and nonasthmatic groups.

Phadiatop analysis

Phadiator (Pharmacia Diagnostics AB, Uppsala, Sweden) is a screening test demonstrating the presence of allergen-specific IgE antibodies to one or several of the 10 most common airborne allergens. Since these allergens are collectively bound to one cellulose RAST disk, the analysis provides only a positive or negative result without any definition of the allergen specificity of the IgE antibodies.

Total IgE analysis

Total IgE analysis was performed as an inhibition assay, PRIST (Pharmacia Diagnostics AB, Sweden), according to the manufacturer's recommendation.

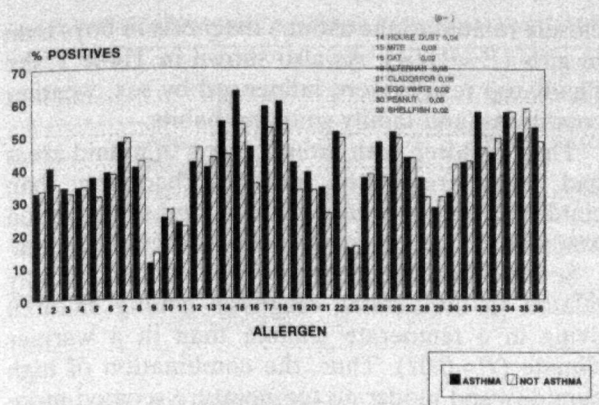


Fig. 2. Frequency of IgE binding to MAST pipette allergens causing recorded voltage of >0.18 V in 171 asthmatic and nonasthmatic children. Allergens included in MAST allergy test were as follows. Tree pollen: 1) box elder, 2) cottonwood/willow/aspen mix, 3) elm mix, 4) *Melaleuca*, 5) oak mix; grass pollen: 6) grass mix, 7) Bahia, 8) Bermuda; weed pollen: 9) chenopod mix II, 10) pigweed mix, 11) ragweed mix I, 12) *Rumex* mix, 13) sage mix; dust/mites/insects/epithelial: 14) house dust, 15) mite (*D. farinae*), 16) cat, 17) dog, 18) cockroach mix; molds: 19) *Alternaria*, 20) *Candida*, 21) *Cladosporium*; food: 22) almond, 23) cereal mix, 24) corn, 25) egg white, 26) garlic, 27) milk, 28) onion mix, 29) orange, 30) peanut, 31) potato, 32) shellfish, 33) soybean, 34) vegetable mix, 35) wheat, 36) yeast.

Statistics

The data were analyzed with the Epi Info, version 6, Public Domain Software for Epidemiology and Disease Surveillance (Centers for Disease Control, Epidemiology Program, Atlanta, GA, USA) and the SPSS Package. The statistical analyses comprised the chi-square test with the Yates continuity correction and Student's *t*.

Results

IgE MAST analyses

The spectrum and magnitude of positive reactions in the sample of asthmatics and nonasthmatics are shown in Fig. 1, where the results are expressed as the mean voltage for each allergen in the asthmatic and nonasthmatic groups separately.

The highest mean of IgE binding to airborne allergens in both groups was to *Dermatophagoides farinae*, followed by dog, house dust, cockroach, *Rumex* mix, Bermuda grass pollen, and Bahia pollen. Considerable IgE binding was obtained with shellfish, vegetable mix, almond, garlic, and wheat. The reactivities were consistently higher in asthmatics than in nonasthmatics, although the differences were not statistically significant for any particular allergen.

The results of the pipette analyses, when allergen-specific IgE concentrations down to a chemiluminescence signal of low level (>0.18 V) were included as positives, are shown in Fig. 2.

For most allergens, the presence of specific IgE antibodies was higher in the asthmatic, than in the nonasthmatic group, reaching 60% for *D. farinae* and cockroach, and almost the same level for dog allergen. The differences between asthmatics and nonasthmatics were statistically significant for house dust and *D. farinae* ($P < 0.04$). Similar differences ($P < 0.05$) were found for egg white, peanut, and shellfish. On the other hand, positive reactions to grass pollen were slightly but not significantly more common in nonasthmatics than in asthmatics.

Table 1. Effect on Phadiatop results in asthmatic and nonasthmatic children by sex, age, climate, domicile, and smoking in family ($n=277$); results shown as percentages

		Positive Phadiatop			Asthma		Not asthma		P
		n	n	%	n	%	n	%	
Sex	M	148	91	61.5	70	70	78	59.8	0.04
	F	129	56	43.4	66	47	63	39.7	0.4
Age	<10 years	146	77	52.7	68	55.9	78	50	0.4
	≥10 years	129	70	53.3	66	63.6	63	44.4	0.02
Rain precipitation	<2000 mm	198	98	49.5	100	45	98	45.9	0.3
	≥2000 mm	79	49	62	36	75	43	51.1	0.03
Temperature	<25°C	193	111	54.7	92	63	111	47.7	0.02
	≥25°C	74	36	48.6	44	50	30	46.7	0.7
Residence	Urban	126	70	55.5	60	63.3	66	48.5	0.09
	Rural	151	77	51	76	55.3	75	46.7	0.2
	Mother smokes	254	136	53.4	119	59.6	135	48.1	0.05
	Does not smoke	23	11	47.8	17	52.9	6	33.3	0.4
Smoking habits	Father smokes	176	101	57.4	83	62.7	93	52.7	0.18
	Does not smoke	101	49	48.5	53	52.8	48	37.5	0.12
	Family smokes	68	41	60.3	46	67.4	22	45.5	0.08
	Does not smoke	209	106	50.7	90	54.4	119	47.9	0.3

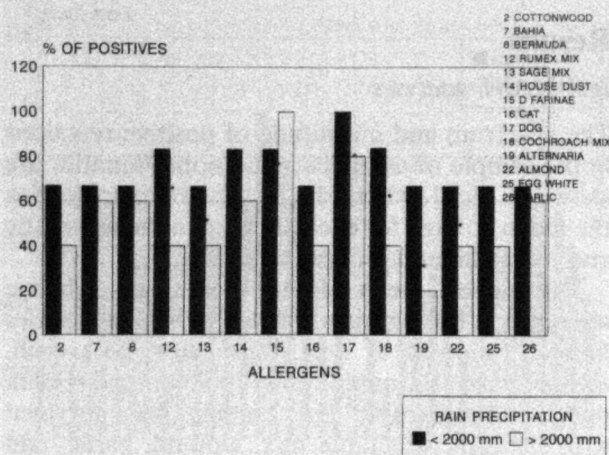


Fig. 3. Frequency of IgE binding to MAST pipette allergens in 44 Phadiatop-positive sera from children living in areas with rain precipitation <2000 or >2000 mm (average precipitation/year). $P < 0.05$ (*); other $P = \text{NS}$. Mean $V > 0.18$.

When the comparison of IgE binding in asthmatics and nonasthmatics was made with stronger criteria for positivity (>0.67 V), the differences between the asthmatics and nonasthmatics became significant for cockroach, chenopods, egg white, and other food allergens ($P < 0.02$), but no longer reached significance for house dust, *D. farinae*, and cat allergen. *D. farinae* was again the most prevalent allergen, causing positive reactions in 40% of the asthmatics. Children living in rural areas were more often sensitized to such allergens as cat and cockroach than children living in urban areas (cat, $P = 0.01$; cockroach, $P = 0.01$); furthermore, house dust ($P < 0.01$), mite ($P < 0.01$), cat ($P = 0.01$), dog ($P < 0.01$), cockroach ($P < 0.01$), *Alternaria* ($P < 0.01$), and egg white ($P = 0.05$) more often caused an IgE response in asthmatic boys than girls.

No significant difference in the prevalence of sensitization to the 36 allergens tested was found in asthmatic children in comparing those younger and older than 10 years.

Phadiatop analyses

Among the sera tested with Phadiatop, 50.4% had positive results and 49.6% negative. The test was positive in 59.8% of the 136 asthmatic children and in 39.7% of the 141 nonasthmatics ($P = 0.006$). The difference was more pronounced in children older than 10 years, as shown in Table 1.

In this subgroup, Phadiatop was positive in 63.3% of the asthmatics and 44.4% of the nonasthmatics ($P = 0.02$).

Regardless of being asthmatic or nonasthmatic, boys had a positive Phadiatop test more often than girls ($P = 0.04$), and a positive Phadiatop was more

closely related to the asthma diagnosis in boys than in girls ($P = 0.004$). As also shown in Table 1, the Phadiatop results were influenced by sex, weather conditions, and family smoking habits.

Thus, children with asthma living in humid areas had more frequently positive Phadiatop than children living in a drier climate, and sensitization was more closely related to the asthma diagnosis.

Sensitization was also more prevalent and closely related to the asthma diagnosis among children living in a temperate climate than in a warmer climate ($P = 0.02$). Thus, the combination of high humidity and moderate temperature seemed more frequently to facilitate sensitization, particularly among the asthmatics. No significant differences in the prevalence of positive Phadiatop were found in children from families with smoking members, although there was a tendency for such children to be more often sensitized.

Those children with asthma having mothers smoking more than five cigarettes daily had more often positive Phadiatop results (59.6%) than those in the nonasthma group (48.1%, $P = 0.05$).

Differences in breast-feeding habits during infancy did not influence the results (78% of the asthmatic and 76% of the nonasthmatic children were breast-fed exclusively during the first 2 months). Children who had been given an allergy diagnosis by a physician were more often Phadiatop positive (63%) than children who had not been given such a diagnosis (36.8%, $P = 0.01$).

According to the parents, certain conditions were especially likely to influence the exacerbation of asthma symptoms in their children. Confirmation of these triggering factors was more common in the positive Phadiatop group than in the negative group; for example, upper respiratory tract infections ($P < 0.01$), dust ($P < 0.01$), humidity ($P < 0.05$), and allergy ($P < 0.01$).

Those children with asthma ($P = 0.0001$) and no asthma ($P = 0.02$) and positive MAST had more often positive Phadiatop than those with negative MAST.

MAST pipette results in 44 Phadiatop-positive asthmatic children indicated, as seen in Fig. 3, that sensitization to *D. farinae* was more frequent (almost 100%) among children living in humid areas (rain precipitation >2000 mm) than in less humid regions. In the latter areas, sensitization to *Rumex* and sage as well as to dog, cockroach, and *Alternaria*, was significantly more prevalent than in the more humid areas ($P < 0.05$). It also appeared that asthmatic children below the age of 10 years were significantly more often sensitized to house dust, *D. farinae*, cat, and *Alternaria* than asthmatics older than 10 years of age ($P < 0.05$). On the other hand, older asthmatics were more frequently

Table 2. Total IgE in asthmatic and nonasthmatic children; results shown in kU/l

		<i>n</i>	Total IgE mean	SD	<i>P</i> value
Asthmatics	Asthma	162	372.2	330.7	0.00001
	Not asthma	158	249.1	298.2	
	Positive Phadiatop	204	487.1	328.4	0.00001
	Negative Phadiatop	208	113.3	147.5	
	Positive Phadiatop	100	519.9	322.8	0.00001
	Negative Phadiatop	62	133.9	161.8	
Nonasthmatics	Positive Phadiatop	74	428.3	329.5	0.00001
	Negative Phadiatop	84	91.2	137.3	

sensitized to cottonwood, Bahia pollen, and almond than the younger ones ($P=0.05$).

Total IgE analyses

The total IgE concentration analyzed in 320 serum samples was significantly higher among asthmatic children (372.2 kU/l) than among nonasthmatic children (249.1 kU/l, $P=0.00001$) (Table 2). Children with a positive Phadiatop test had significantly higher total IgE (487.1) than those with negative Phadiatop, whether asthmatic (133.9) or nonasthmatic (91.2, $P=0.00001$).

Discussion

This study shows a very high prevalence of atopic sensitization in Costa Rica, both in asthmatic and in nonasthmatic schoolchildren. The cohort studied had been randomly selected in order to represent a relevant sample from our previous epidemiologic study, which showed a prevalence of asthma in Costa Rican schoolchildren of 23% (6). This prevalence figure was based on a classification dividing the children simply into asthmatics and nonasthmatics.

In 60% and 45% of these two groups of schoolchildren, respectively, we could demonstrate circulating specific IgE antibodies to one or several of the 9–10 most common airborne allergens by using the Phadiatop test. Since bronchial asthma, although highly prevalent in Costa Rica, represents only a part of the atopic disease spectrum, it is not surprising that specific IgE antibodies were also frequently demonstrated in the nonasthma group. In fact, manifestations of atopic diseases other than asthma appeared in almost 40% of the children classified as nonasthmatics.

Several European studies have demonstrated that a positive Phadiatop test is highly predictive of atopic disease (14, 15). It is also suggested that the selection of the Phadiatop allergens has a global relevance for allergy screening with Phadiatop, although the test does not give an allergen-specific

result. We found the MAST pipette analyses more laborious and technically difficult to perform than the Phadiatop. On the other hand, the MAST test offered the opportunity, by one single analysis, to detect specifically the sensitizing allergens and to compare different sensitization patterns in subgroups of our sample. The clinical relevance of the MAST pipette test has been less extensively documented. However, one study has shown that data obtained with MAST pipettes matched bronchial provocation tests more closely than RAST and to the same degree as skin prick tests (16), and the statistical problems in assessing the true value of this test system have been reviewed (17). Skin testing was not a feasible option at the time of this study. It seems likely that the two *in vitro* methods used in this study have furnished information quite comparable with extensive prick testing.

In many Latin-American countries, the most common allergen appears to be the house-dust mite (18–20). It has been shown that sensitization to *D. pteronyssinus* varies from 60.7% to 91.2% and to *D. farinae* from 53.3% to 97.2% in certain areas in Latin America, at sea level as well as at high altitudes (18). Sensitization to *D. farinae* in this study, representative of all Costa Rican schoolchildren, was around 50%, suggesting a nationwide problem with mite allergy.

Unfortunately, the panel of MAST pipette allergens did not include *D. pteronyssinus*, which has been reported to be the most prevalent mite allergen in Costa Rica (20). On the other hand, *D. farinae* has been found to cross-react to a certain extent with *D. pteronyssinus*, and these species usually occur together. Our finding of a mite allergen as the most important sensitizing allergen in Costa Rica is likely to be of clinical importance and related to the high asthma prevalence. The prevalence of sensitization might have been even higher if we had included a *D. pteronyssinus*-specific test. Results from analyses of bed dust in the study areas shows mite-allergen concentrations to be generally very high (28).

Second to *D. farinae*, cockroach appeared to be the most common allergen in our study, with 33% positives in asthmatics and 22% in nonasthmatics ($P=0.02$). Cockroach debris is a common source of indoor allergen, particularly in lower socioeconomic groups and in tropical countries (9, 21), and Costa Rica offers both these conditions.

The importance of sensitization to food allergens for the causation of asthma has been discussed, but is mostly restricted to case reports (22–24). The results indicating food sensitization in this study population may be related to other atopic mani-

festations than asthma. They can also be due to cross-reactivity between food and pollen allergens.

Among the mold allergens, *Alternaria* was the most prevalent in the studied population. Some reports have shown the relation between this allergen and the severity of asthma. Other investigations suggest that cat and dog allergens are important causes of asthma in children (25, 26). In this study, dog allergen had more often sensitized children than cat allergen, possibly because dogs are more common indoor pets than cats in Costa Rica.

In a country such as Costa Rica, situated in the tropics with a high humidity and a multitude of plants and flowers, the prolonged exposure to outdoor allergenic pollen might produce sensitization and play a certain role in the appearance of asthmatic symptoms. However, pollen allergens mainly cause eye and nose symptoms, and in spite of a relatively high prevalence of sensitization to Bahia and Bermuda grass, specific IgE antibodies to these pollen allergens were as common among asthmatics as among nonasthmatics.

Sex differences in the prevalence and natural history of childhood asthma have been described (27). In our study, asthmatic boys were more often sensitized to house dust, mite, cat, dog, cockroach, *Alternaria*, and egg white than girls.

But we did not find any difference in sensitization to the allergens tested in asthmatics younger or older than 10 years of age.

We found more Phadiatop positives among asthmatics than nonasthmatics in children older than 10 years of age. Regardless of being asthmatic or nonasthmatic, boys had a positive Phadiatop test more often than girls.

Children living in rural areas were more often sensitized to cat and cockroach allergens than children living in urban areas, probably due to the predominance of these types of animals.

Children with asthma living in humid areas more frequently had positive Phadiatop than children living in a drier climate, and sensitization was also more prevalent and closely related to the asthma diagnosis among children living in a humid and temperate climate than in a warmer climate ($P=0.02$). Thus, the combination of high humidity and moderate temperature seemed more frequently to facilitate sensitization, particularly among the asthmatics.

Thus, it is clear from our study that Phadiatop results were influenced by sex, weather conditions, and family smoking habits.

In conclusion, our data indicate that allergens that may be the cause of the high prevalence of bronchial asthma in Costa Rican schoolchildren are probably produced indoors by mites, cock-

roaches, and dogs, in that order. For some reason, male sex and living in humid, but temperate areas of Costa Rica facilitate sensitization. As expected, the presence of smoking mothers also does this.

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