Children at risk for asthma: Home allergen levels, lymphocyte proliferation, and wheeze

Patricia W. Finn, MD[a,d] Jacques O. Boudreau, BS,[b] Hongzhen He, MD,[a] Yunsheng Wang, MD,[a] Martin D. Chapman, MD,[e] Carey Vincent, PhD,[c] Harriet A. Burge, PhD,[c] Scott T. Weiss, MD, MSc,[c,d] David L. Perkins, MD, PhD,[b] and Diane R. Gold, MD, MPH[c,d] *Boston, Mass, and Charlottesville, Va*

Background: Allergic asthma is a common childhood disease. Although T-lymphocyte activation plays a critical role in allergic asthma, the environmental factors promoting lymphocyte activation in children are not well defined.
Objective: In a cohort of children at risk for asthma (n = 114), we determined whether the levels of cockroach (Bla g 1 or 2), house dust mite (Der f 1), and cat allergen (Fel d 1) in the home during infancy was associated with subsequent allergen-specific lymphocyte proliferation in later life.
Methods: Dust samples from multiple sites in the home were collected at 3 months of age and were measured for allergen levels. Serial questionnaires were applied. At a median age of 2 years, PBMCs were isolated and lymphocyte proliferation to the home allergens and PHA was determined.
Results: Increased lymphocyte proliferative responses to Bla g 2 were associated with higher home levels of Bla g 1 or 2 (*P* for trend with kitchen Bla g levels = .011), in analyses adjusting for cold in the past week. Proliferative responses to Der f 1 were higher in homes with family room levels of Der f 1 ≥10 μg/g dust than in homes with Der f 1 <2 μg/g, but differences were not significant in analyses adjusting for cold (*P* = .15). Repeated wheeze in the first 2 years of life was associated with increased allergen-specific and PHA proliferative responses.
Conclusion: Early-life cockroach allergen exposure at 3 months of age predicts allergen-specific lymphocyte proliferative responses at a median of 2 years of age.
(J Allergy Clin Immunol 2000;105:933-42.)

Key words: *Children, T lymphocytes, proliferation, home allergen levels, allergy, asthma, cockroach, Bla g 2, Der f 1, Fel d 1*

Abbreviations used
Cyt C: Pigeon cytochrome C
LRI: Lower respiratory tract infection
OVA: Chicken ovalbumin
SI: Stimulation index
URI: Upper respiratory tract infection

In the past 40 years asthma has become the most prevalent chronic disease of childhood in developed countries.[1,2] Recent studies suggest that most asthmatic patients are diagnosed by the age of 6 years, with symptoms first occurring during infancy and early childhood.[3,4] Allergy is known to play a significant role in childhood asthma in Western countries. Little is known about the cellular mechanisms promoting the development of the allergic phenotype. T lymphocytes are critical for the initiation and maintenance of the allergic and asthmatic inflammatory response.[5] Induction of T-lymphocyte activation leads to a cascade of responses, including cytokine and IgE production, and inflammation. The T-lymphocyte activation that leads to the asthmatic phenotype likely occurs in early childhood under the influence of a myriad of factors. Understanding the mechanisms that promote childhood asthma may help to identify individuals at risk for development of asthma and lead to the development of novel strategies to prevent this disorder.

Early expression of the asthmatic phenotype represents contributions from a variety of genetic, developmental, immunologic, and environmental factors.[5] T-lymphocyte function itself is strongly influenced by genetic factors.[6] The asthmatic phenotype, as related to T-lymphocyte activation, is modulated by environmental factors, including viral infection, industrial air pollutants, maternal smoking, breast-feeding, and early allergen exposure.[3,7-9] Although asthma symptoms in allergic adults are worsened with exposure to inhaled allergens,[10,11] the role of inhaled allergens in developing asthma is poorly understood. More than 80% of childhood asthmatics exhibit allergies to one or more allergens.[3] Prospective studies of early allergen exposure and subsequent allergic responses of children have been primarily limited to the analysis of house dust mite allergens.[12-15] An examination of other allergens found a correlation between home levels of cockroach allergen and repeated wheeze in a population of 1-year-old children with a family history of asthma or atopy.[16]

From the [a]Respiratory and Critical Care Division, the [b]Renal Division, and the [c]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, the [d]Pulmonary Division, Beth Israel Hospital, Boston, Mass, and the [e]Asthma and Allergic Diseases Center, University of Virginia, Charlottesville, Va.
Supported by National Institutes of Health grants No. AIEHS35786 and AIEHS45007 and by Astra USA.
Received for publication Sept 8, 1999; revised Feb 16, 2000; accepted for publication Feb 16, 2000.
Reprint requests: Patricia W. Finn, MD, Respiratory and Critical Care Division, Brigham and Women's Hospital, 75 Francis St, Boston, MA 02115.

0091-6749/2000 $12.00 + 0 **1/1/106546**
doi:10.1067/mai.2000.106546

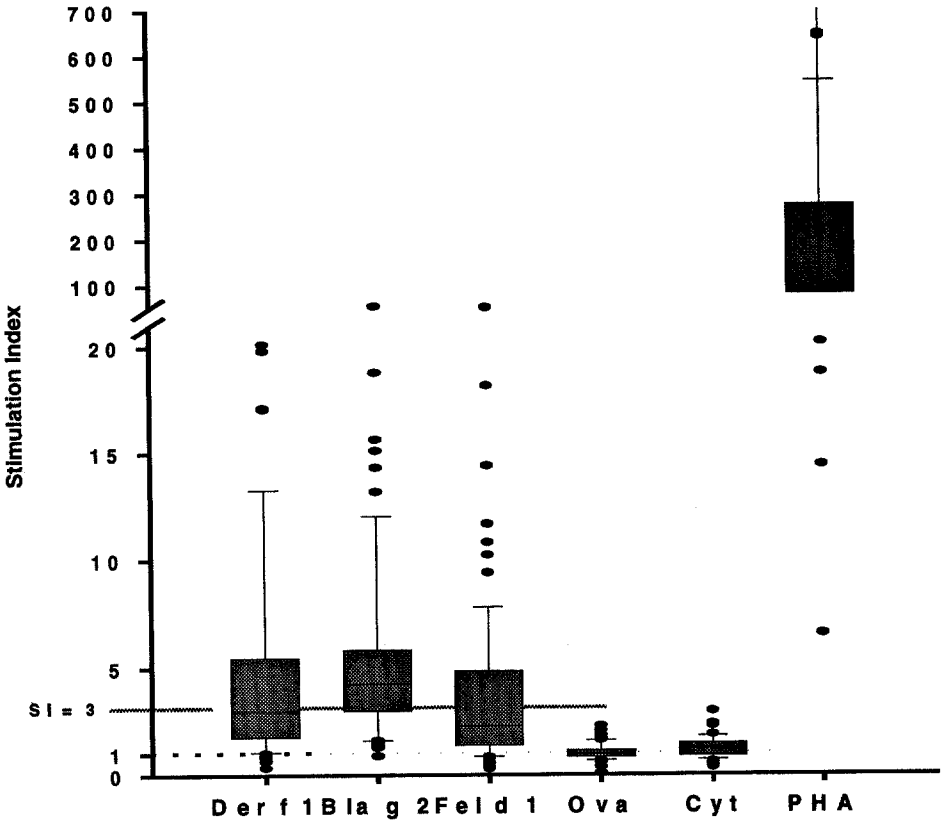

FIG 1. Lymphocyte proliferative response after stimulation with antigens Bla g 2, Der f 1, Fel d 1, OVA, Cyt C, and PHA. PBMCs were obtained from blood samples collected between the ages of 18 and 32 months (n = 114). Cells were obtained by Ficoll-Hypaque separation and were cultured in the presence or absence of Bla g 2, Der f 1, Fel d 1, OVA, Cyt C, and PHA for 80 hours. Proliferation was measured by calculating the SI representing the ratio of the mean tritiated thymidine uptake of allergen-stimulated to unstimulated media alone cells. An SI >3 is a positive response. The median values are shown (Bla g 2: 4.22; Der f 1: 3.15; Fel d 1: 2/40; OVA: 1.01; Cyt C: 1.12; PHA: 153). The response to PHA was indexed into a high and low group about the median SI = 153.

Our study investigates potential cellular mechanisms linking environmental factors and T-lymphocyte activation. We examine the impact of early aeroallergen exposure, namely, levels of cockroach (Bla g 1 or 2), house dust mite (Der f 1), and cat (Fel d 1) on lymphocyte proliferation in a cohort of children at risk for allergic asthma. We postulate that aeroallergen exposure effects the development of later immune responses.

METHODS

Description of cohort

Participants in this longitudinal study were selected as previously described.[17] In brief, families with a parental history of asthma or allergy were enrolled in a birth cohort study designed to examine the effects of allergen exposure in early life on the development of asthma. Mothers in the greater Boston metropolitan area delivered at a large Boston hospital were screened with the following questions: (1) Have you ever had asthma, hay fever, or allergies? and (2) Has the biologic father of your child ever had asthma, hay fever, or allergies? Mothers responding "yes" were asked to complete a screening questionnaire. One month after birth, the mothers were contacted by phone and asked to respond to a second questionnaire. Families were not approached if the index child was premature, had a major congenital anomaly, or was in the neonatal intensive care unit or if the mother was less than 18 years old or could not speak English or Spanish. Those children who met the inclusion criteria and whose mothers expressed an interest were accepted into the study. Of the 1405 mothers initially screened from September 1994 to June 1996, 505 children were enrolled in the study. Reasons for exclusion were plans to move within the next year (39% of those screened), reluctance to participate (45%), lost to follow-up (15%), and other (2%).

Every 2 months an additional telephone questionnaire was administered to the primary care provider. In this questionnaire, information was obtained regarding changes of the physical condition of the child and various home characteristics.

This study was approved by the Brigham and Women's Hospital Human Research Committee. Informed consent was obtained from the parents for blood collection and longitudinal follow-up.

TABLE I. Home allergen levels as univariate predictors of allergen-specific lymphocyte response

	Allergen-specific lymphocyte proliferation >3*				
	No.	%	Relative risk	95% Confidence limits	*P* for trend
Home Bla g 1 or 2 levels (U/g)					
Kitchen*					
Low (<0.05) = 0	56	64	1.00		
Medium (0.05 to <2)	45	67	1.23†	†	
High (≥2)	11	100	1.56†	†	.013‡
Family room					
Low (<0.05) = 0	77	70	1.00		
Medium (0.05 to <2)	25	84	1.20†	†	
High (≥2)	4	100	1.43†	†	.079
Home maximum					
Low (<0.05) = 0	43	63	1.00		
Medium (0.05 to <2)	54	78	1.24	0.94-1.62	
High (≥2)	16	88	1.39†	1.04-1.87	.036‡
Home Der f 1 levels (μg/g)					
Family room					
Low (<2) = 0	63	49	1.00		
Medium (2 to <10)	26	62	1.25	0.84-1.85	
High (≥10)	22	68	1.39	0.95-2.03	.096
Home maximum					
Low (<2) = 0	45	58	1.00		
Medium (2 to <10)	24	42	0.72	0.42-1.23	
High (≥10)	45	62	1.08	0.77-1.51	.671
Home Fel d 1 levels (μg/g)					
Family room					
Low (<1) = 0	40	38	1.00		
Medium (1 to <8)	32	47	1.25	0.73-2.15	
High (≥8)	37	35	0.94	0.52-1.70	.849
Home maximum					
Low (<1) = 0	37	41	1.00		
Medium (1 to <8)	38	42	1.04	0.61-1.78	
High (≥8)	40	35	0.86	0.49-1.53	.612

Unless otherwise stated, variables are categorical, with category of comparison labeled as 0.
*Lymphocyte proliferative response to stimulation with Bla g 2, Der f 1, or Fel d 1.
†The category was found to be an ideal predictor and unable to produce statistical univariate values for *P* or 95% confidence intervals. Relative risks were calculated in comparison to the reference group according to the following formula: Relative risk = (Population percent of children with SI >3)$_{\text{indexed category}}$/(Population percent of children with SI >3)$_{\text{reference group}}$.
‡$P < .050$.

Home visit

When the child was approximately 3 months old, a home visit was made, and dust samples were collected and examined for the presence of several allergens, as previously described.[18] In brief, dust samples were collected by vacuuming with a modified Eureka Mighty-Mite in a standardized fashion for each individual at 4 sites in the home: the floor around the baby's bed, the baby's bed, the family room, and the kitchen. If a rug was present in any area, half the time of collection was spent sampling the rug.

After vacuuming, the dust was immediately placed into an airtight bag, weighed, and sifted through a 425-μm mesh sieve. A portion of the sample was extracted (1:10 [wt/vol] in 0.1 mol/L ammonium bicarbonate buffer) and assayed for allergens including house dust mite (Der f 1), cockroach (Bla g 1 or 2), and cat (Fel d 1). Prioritization of allergen quantification was based on the estimated prevalence of allergen in a given room location. For limited samples taken at the baby's floor, baby's bed, family room, and parents' bed, house Der f 1 was quantified first, followed by Fel d 1 and then Bla g 1 or 2. For limited samples obtained in the kitchen, Bla g 1 or 2 were quantified first, followed by Fel d 1 and Der f 1. Quantification of allergen was performed by 2-site monoclonal and polyclonal antibody immunoassays as previously described.[19-22] Allergen concentrations were reported as micrograms of allergen per gram of dust except for Bla g 1 or 2, which were reported as units per gram of dust. In addition to the levels measured at the 4 previously described locations, a home-maximum value of Bla g 1, Bla g 2, Der f 1, and Fel d 1 was determined for each child. This variable represents the highest allergen level obtained in the 4 locations.

Lymphocyte proliferative responses

On the basis of measured home allergen levels, values in the highest or lowest quartiles for any one of the 3 allergens, children were screened for a further questions and peripheral blood analysis. From the 505 children, 114 were selected on the basis of home allergen levels. At 2 years of age (range 18 to 32 months) during routine lead-level testing, additional blood was drawn for allergen-specific lymphocyte proliferation. At the time of the blood draw, a questionnaire was administered to determine the presence of an upper respiratory infection (URI) within the previous week.

With use of Ficoll-Hypaque centrifugation, PBMCs were isolated as previously described.[23] PBMCs were immediately processed and not cryopreserved. Cells were separated into 5×10^5 cell aliquots and incubated in quadruplicate in a 96-well microtiter plate with 100 μL of media alone, media with PHA (10 μg/mL), or with the fol-

TABLE II. Univariate predictors of increased lymphocyte proliferative responses at 2 years of age: sex, history of LRI, age, race, and wheeze history

	Allergen-specific proliferative response							
	Bla g 2				Der f 1			
	No.	SI >3 (%)	Relative risk	Statistical significance	No.	SI >3 (%)	Relative risk	Statistical significance
Sex								
Male = 0	70	71	1.000		70	53	1.000	
Female	43	77	1.074	$P = .525$	44	61	1.161	$P = .364$
Has your child had an LRI before?								
No = 0	63	69	1.000		69	52	1.000	
Yes	45	80	1.157	$P = .184$	45	62	1.193	$P = .282$
Age at time of first blood draw								
<26 mo = 0	69	75	1.000		70	49	1.000	
≥26 mo	44	70	0.935	$P = .573$	44	68	1.404*	$P = .035$
Race								
White = 0	83	71	1.000		84	60	1.000	
Black and Hispanic	21	71	—	—	21	43	0.720	$P = .220$
Asian and other	9	100	†	†	9	56	0.933	$P = .825$
Has your child ever wheezed?								
No = 0	59	68	1.000		59	49	1.000	
Yes	54	80	1.175	$P = .155$	55	64	1.295	$P = .122$
Has your child ever had repeated wheeze?								
No = 0	80	70	1.000		81	51	1.000	
Yes	33	82	1.169	P = .156	33	71	1.377*	P = .044

Unless otherwise stated, variables are categorical, with category of comparison labeled as 0.
*P < .050.
†The category was found to be an ideal predictor and unable to produce statistical results.
‡P < .075.

TABLE III. Upper respiratory illness/symptoms in the week prior to blood draw as a predictor of lymphocyte proliferative responses

	Allergen-specific proliferative response							
	Bla g 2				Der f 1			
	No.	SI >3 (%)	Relative risk	Statistical significance	No.	SI >3 (%)	Relative risk	Statistical significance
Did your child have a cold in the past week?								
No = 0	84	80	1.000		85	62	1.000	
Yes	26	54	0.675*	$P = .038$	26	39	1.617†	$P = .065$
Did your child have a cough in the past week?								
No = 0	85	77	1.000		86	63	1.000	
Yes	27	63	0.823	$P = .223$	27	37	0.590*	$P = .046$
Did your child have a cough or cole in the past week?								
No = 0	78	80	1.000		79	62	1.000	
Yes	35	60	0.755†	$P = .060$	35	43	0.691	$P = .084$
Did your child have a fever in the past week?								
No = 0	98	76	1.000		99	57	1.000	
Yes	14	57	0.757	$P = .243$	14	57	1.010	$P = .967$
Did your child have a runny nose in the past week?								
No = 0	66	74	1.000		66	64	1.000	
Yes	47	72	0.974	$P = .823$	48	46	0.720†	$P = .072$

Unless otherwise stated, variables are categorical, with category of comparison labeled as 0.
*$P < .050$.
†$P < .075$.

Allergen-specific proliferative response				PHA proliferative response			
Fel d 1							
No.	SI >3 (%)	Relative risk	Statistical significance	No.	SI >3 (%)	Relative risk	Statistical significance
71	41	1.000		71	53	1.000	
44	37	0.890	$P = .636$	44	58	1.092	$P = .645$
69	41	1.000		69	45	1.000	
46	37	0.911	$P = .699$	46	57	1.258	$P = .216$
71	39	1.000		71	42	1.000	
44	39	0.980	$P = .932$	44	61	1.452*	$P = .042$
85	42	1.000		85	48	1.000	
21	23	0.562	$P = .161$	21	43	0.941	$P = .811$
9	44	1.049	$P = .903$	9	44	0.879	$P = .739$
59	36	1.000		59	41	1.000	
56	43	1.204	$P = .426$	56	59	1.449‡	$P = .055$
82	35	1.000		82	43	1.000	
33	48	1.371	P = .177	33	67	1.562*	P = .012

Allergen-specific proliferative response				PHA proliferative response			
Fel d 1							
No.	SI >3 (%)	Relative risk	Statistical significance	No.	SI >3 (%)	Relative risk	Statistical significance
86	44	1.000		86	53	1.000	
26	23	0.522	$P = .086$	26	42	0.791	$P = .34$
87	44	1.000		87	48	1.000	
27	26	0.594	$P = .0133$	27	56	1.151	$P = .49$
80	44	1.000		80	51	1.000	
35	29	0.653	$P = .150$	35	46	0.892	$P = .59$
100	40	1.000		100	51	1.000	
14	36	0.893	$P = .765$	14	43	0.840	$P = .59$
67	42	1.000		67	55	1.000	
48	35	0.847	$P = .495$	48	42	0.755	$P = .16$

lowing antigens: Der f 1 (30 μg/mL), Bla g 2 (30 μg/mL), Fel d 1 (1000 U/mL), chicken ovalbumin (OVA) (30 μg/mL), and pigeon cytochrome C (Cyt C) (30 μg/mL). Optimal concentrations of the allergens had been determined in a dose-response analysis (data not shown). After 72 hours, 1 μCi tritiated thymidine was added to each well. After incubation for an additional 8 hours, the cells were harvested and tritiated thymidine uptake was determined by β-counting. Proliferation was quantified by calculating the stimulation index (SI) for each antigen with the following formula: SI = (Mean value of tritiated thymidine uptake for stimulated samples)/(Mean value tritiated thymidine uptake for unstimulated samples).

Definition of predictor variables

As in previous analyses, allergen levels were categorized as binary indices at which associations between exposure and sensitization or development of allergy-related repeated wheeze have been determined.[12,13,15,24,25] Levels in the various house locations and home maximum allergen values were assessed as predictors of allergen-specific proliferative response. Der f 1 levels were categorized into the following indices: high (≥10 μg/g dust), medium (2 to <10 μg/g), low (<2 μg/g), no dust. Because of limited amounts of dust collected in the various locations and the priorities of allergen measurements as described above, the variable Bla g 1 or 2 was created to represent the maximum level of the species Bla g 1 or 2 measured at each location. Bla g 1 or 2 levels were categorized into the following indices: high (≥2 U/g), medium (0.05 to <2 U/g), low (<0.05 U/g), no dust. Fel d 1 levels were categorized into the following indices: high (≥8 μg/g), medium (1 to <8 μg/g), low (<1 μg/g), no dust. Values that were found to be above the upper levels of detection for the assay were indexed as high. Values that were found to be below the levels of detection were indexed as low.

For each child, the presence of a URI within the week before blood draw was determined according to a parental questionnaire. Lower respiratory illness (LRI) was defined as physician-diagnosed croup, bronchitis, bronchiolitis, or pneumonia.[26] Presence of wheeze was determined by parental reporting. Episodes of wheeze were monitored every 2 months during the administered phone questionnaire as described above. Repeated wheeze was defined as 2 or more reports of wheeze up to the time of the blood draw. The race/ethnicity of the child was defined according to parental reporting. If both parents were white, the child was classified as being white. If either parent was black, the child was classified being as black. If no parent was black but at least one parent was Hispanic, the child was classified as being Hispanic. If no parent was black or Hispanic but at least one parent was Asian, the child was classified as being Asian. The age of the child was determined at the time of blood draw.

Definition of outcome variable

The lymphocyte proliferative response was measured by calculating the SI as described above. Each sample was assayed in quadruplicate and a mean value was calculated. The SI response was indexed into binary variables of high and low responses. Allergen-specific proliferative responses with an SI >3 were categorized as positive responses.

Statistical methods

Univariate and multivariate associations between predictor variables and allergen-specific proliferative response were made with use of SAS statistical software (SAS Institute, Cary, NC). Univariate associations between indexed predictor variables and indexed proliferative responses were modeled with (N × 2) contingency tables then calculated for relative risk with the logit method. Multivariate associations were modeled with use of the logit method. Log relative risk values were determined directly with the use of SAS PROC GENMOD (SAS Institute, 1997), with log link and binomial variance function.

RESULTS

Increased lymphocyte proliferation in the presence of Der f 1, Bla g 2, and Fel d 1

To determine the immune response of lymphocytes to allergens assayed in the house, we determined the allergen-specific proliferative response of PBMCs stimulated with documented home allergens. The proliferative response of PBMCs isolated from each child was determined after stimulation with the following antigens: Bla g 2, Der f 1, Fel d 1, OVA, Cyt C, and PHA. The distributions of the SI responses were found to be single tailed in their distribution (Fig 1). Lymphocytes from all children proliferated to the nonspecific mitogen PHA (median 153) but not to the antigens OVA or Cyt C (median 1.01 and 1.12, respectively). OVA and Cyt C, as food and irrelevant antigens, respectively, would not be expected to be present in the home as aeroallergens. In contrast, the previously documented aeroallergens elicited a range of proliferative responses with SI median values as follows: Bla g 2 = 4.22, Der f 1 = 3.15, and Fel d 1 = 2.40. The separation of the PHA-induced proliferative response was indexed into high and low proliferative response groups solely on the basis of the median value of the SI distribution.

Univariate analysis

Home allergen levels as predictors of allergen-specific proliferative response. Increased Bla g 2–specific lymphocyte proliferative responses were associated with higher levels of kitchen or home maximum Bla g 1 or 2 (*P* for trend = .013 and .036, respectively) (Table I). A suggestive association between allergen levels and an increase in allergen-specific proliferation was observed with both Bla g 1 or 2 and Der f 1 in the family room (*P* for trend = .079 and .096, respectively). Lymphocyte proliferative responses to Der f 1 were higher in homes with family room Der f 1 levels ≥10 μg/g than in homes with family room Der f 1 levels <2 μg/g (relative risk 1.39, 95% confidence limits 0.95-2.03). No association was seen with levels of Fel d 1 and the respective Fel d 1–specific proliferative response. Thus early life levels of Bla g 1 or 2 and Der f 1 in the kitchen and family room, respectively, predicted allergen-specific lymphocyte responses at a later time point.

Predictors potentially confounding the allergen-specific lymphocyte response. The following variables were analyzed as being univariate predictors of altered allergen-specific proliferative responses: age at the time of blood draw, race, sex, history of LRI, and repeated wheeze in the first 2 years of life (Table II). Sex, LRI, and race did not predict allergen-specific lymphocyte proliferative responses. Compared with children aged <26 months, children ≥26 months old had an increased proliferative response to Der f 1 and to PHA but not to Bla g 2 or Fel d 1. Athough associations were not always statistically significant, repeated wheeze tended to be associated with a PHA response of SI >153 and with allergen-specific responses of SI >3.

TABLE IV. Multivariate models of allergen levels in several locations in the home as a predictor of allergen-specific lymphocyte proliferative responses adjusted for the presence of a cold in the prior week

	Allergen-specific lymphocyte proliferation SI >3*		
	Relative risk	***P* value**	***P* for trend**
Home Bla g 1 or 2 levels			
Model 1: kitchen			
Low (<0.05 U/g) = 0	1.00		
Medium (0.05 to <2 U/g)	1.16	.239	
High (≥2 U/g)	999†	†	.011‡
Cold in the past week?			
No = 0	1.00		
Yes	0.72	.099	
Model 2: family room			
Low (<0.05 U/g) = 0	1.00		
Medium (0.05 to <2 U/g)	1.07	.618	
High (≥2 U/g)	999†	†	.156
Cold in the past week?			
No = 0	1.00		
Yes	0.70	.083	
Model 3: home maximum			
Low (<0.05 U/g) = 0	1.00		
Medium (0.05 to <2 U/g)	1.16	.267	
High (≥2 U/g)	1.31	.052§	.049‡
Cold in the past week?			
No = 0	1.00		
Yes	0.71	.072§	
Home Der f 1 levels			
Model 4: family room			
Low (<2 μg/g) = 0	1.00		
Medium (2 to <10 μg/g)	1.21	.343	
High (≥10 μg/g)	1.31	.149	.168
Cold in the past week?			
No = 0	1.00		
Yes	0.59	.064§	
Model 5: home maximum			
Low (<2 μg/g) = 0	1.00		
Medium (2 to <10 μg/g)	0.72	.202	
High (≥10 μg/g)	0.97	.863	.966
Cold in the past week?			
No = 0	1.00		
Yes	0.62	.064§	

Unless otherwise stated, variables are categorical, with category of comparison labeled as 0.
*Lymphocyte proliferative response to stimulation with Bla g 2, Der f 1, or Fel d 1.
†The category was found to be an ideal predictor unable to calculate *P* values and resulted in infinite relative risks.
‡$P < .050$.
§$P < .075$.

The presence of a cold as a predictor of allergen-specific lymphocyte proliferative responses. With use of the cold questionnaire administered at the time of blood draw, parental reported responses suggesting the presence of a URI were analyzed as a univariate predictor of changes in allergen-specific lymphocyte response (Table III). The presence of a cold in the week before blood draw was associated with a reduced allergen-specific lymphocyte response of SI ≤3 to Bla g 2, Der f 1, and Fel d 1 ($P = .038$, .065, and .086, respectively). Although associations were not as strong, cough in the week before blood draw also predicted SI ≤3 for the allergen-specific proliferative responses to Der f 1, Bla g 2, and Fel d 1.

Multivariate analyses

Allergen levels in the home as predictors of allergen-specific proliferation in multivariate models adjusting for the presence of a cold. Multivariate models adjusting for cold in the week before blood collection were also used to test associations between allergen levels measured when children were 3 months old and lymphocyte proliferative responses at a median age of 2 years. Increased Bla g 2–specific lymphocyte proliferative responses were associated with higher levels of kitchen or home maximum Bla g 1 or 2 (P for trend = .011 and .049, respectively) (Table IV). Compared with children from homes with family room levels of Der f 1 <2 μg/g, more children with

Der f 1 family room levels ≥10 μg/g had a Der f 1 lymphocyte proliferative response of SI >3. However, responses did not differ significantly (relative risk 1.31, P = .15) in models adjusting for cold or in models adjusting for both cold and age (relative risk 1.19, P = .31). No association was observed between Fel d 1 levels and lymphocyte proliferative responses to Fel d 1.

These data suggest that a relationship exists between cockroach allergen exposure early in life and allergen-specific lymphocyte proliferative response later in life, independent of the influence of a cold in the week before blood collection.

Repeated wheeze as a predictor of allergen-specific proliferation in multivariate models adjusting for the presence of a cold. After adjustment for cold in the past week, repeated wheeze was associated with a proliferative response of SI >3 to stimulation with Bla g 2 (relative risk 1.21, P = .04), Der f 1 (relative risk 1.40, P = .02), and Fel d 1 (relative risk 1.39, P = .15). After adjustment for cold, repeated wheeze was also associated with a proliferative response of SI >153 to PHA (relative risk 1.47, P = .03).

DISCUSSION

In this investigation, we examined whether environmental exposure to specific allergens in early life would affect later immune functions in a cohort of children with a family history of asthma or allergy. We found that home levels of the cockroach allergen Bla g 1 or 2 at 3 months of age predicted allergen-specific lymphocyte proliferation at a median age of 2 years. T-lymphocyte activation is critical to the induction of an allergic response. Analyses of allergen-specific lymphocyte responses of children, particularly for those less than 6 years old, has been limited either by sample size or allergen selection.[27-30] Here we examine allergen-specific lymphocyte proliferation to multiple aero and food allergens in parallel with measurement of the home aeroallergen levels. The responses to the aeroallergens Bla g 2, Der f 1, and Fel d 1 show a range of allergen-specific proliferation (Fig 1). For the food allergen OVA, there was no proliferative response, similar to other investigations with this age group (Fig 1).[27,29,31] The significantly increased lymphocyte responses to aeroallergens compared with food allergens are consistent with the notion that allergens presented via the airway mucosa may elicit different pathways of T-lymphocyte differentiation than allergens presented across the gastrointestinal epithelium.[32-35] In addition, the range of responses to the different aeroallergens shown also suggests a specificity of T-lymphocyte responses to the type of aeroallergen. During early childhood T-lymphocyte responses are subject to regulatory mechanisms that may be driven by exposure to different environmental allergens.[22,32,36,37] In this population, by the age of 2 years, children have effectively down-regulated T-lymphocyte reactivity to food while reactivity to aeroallergens persists.

The most geographically common home aeroallergens of the Boston area were examined to test whether specific aeroallergens predict immune responses. For each home, dust samples from multiple sites in the home were collected to optimize isolation of the individual areas where the aeroallergens are typically found. Our methods, which examine multiple predominant insect and animal allergens in concert, contrast other studies that have collected a single dust sample from each home comprised of multiple locations.[20,32] With use of these site-specific allergen levels, we show that in a univariate model increasing levels of Bla g 2 in the kitchen and home maximum predicted an increased lymphocyte proliferation (P = .013 and .036, respectively) (Table I). A similar trend could be seen with Bla g 1 or 2 and Der f 1 levels in the family room (P = .079 and .096, respectively).

Highest cockroach allergen levels are typically found in the kitchen and are more representative of the general presence of cockroaches in the home.[20] In contrast, for Der f 1, many locations with upholstery or carpeting contribute to the presence of house dust mites.[38] The absence of dust in the baby's bed resulted in an absence or low level of allergens measured from which no associations with later lymphocyte proliferation were found. In this cohort, the Der f 1 level in the family room consisting of the chair and floor where the baby was nursed provided the strongest association with later Der f 1 proliferation. No associations were seen between Fel d 1 levels and later Fel d 1–specific proliferation. Intrinsic properties of a given aeroallergen contribute to its distribution in the home and may influence subsequent allergen-specific lymphocyte proliferation. Der f 1, Bla g 1, and Bla g 2 are associated with large proteins and only become airborne when disturbed such as when playing on a carpet or sitting on upholstery.[23,39] In contrast, Fel d 1 is associated to a significant extent with smaller particles of less than 5 μm. Consequently, cat allergen remains airborne easily and has been found when sampling undisturbed air.[11] Given the tendency to remain airborne, Fel d 1 is found in a wider range of areas throughout the home and community, even in locations where no cats are kept.[11,40] The constant low-level exposure to cat allergens may result in different immune response to Fel d 1 compared with other aeroallergens.[41,42]

Several factors were investigated as possible contributors to changes in lymphocyte response, including sex, history of LRI, age, race, history of wheeze, and presence of URI (Tables II and III). Changes in allergen-specific proliferation were not associated with sex or race. Although allergen-specific proliferation was only weakly associated with history of LRI (physician-diagnosed bronchitis, bronchiolitis, croup, or pneumonia), others have suggested a possible association between LRI and the development of allergic asthma.[2,7,8] Compared with children aged <26 months, children ≥26 months old had an increased proliferative response to Der f 1 and PHA but not to Bla g 2 or Fel d 1. Previous work suggests that the response to Der f 1 increases with age, perhaps because of increased cumulative exposure to the allergen. If that is the case, we may be overadjusting when we control for age in multivariate models predicting the proliferative response to stimulation

with Der f 1. The relationship between age and proliferative response is likely to be allergen specific.

The report of a cold in the week before blood draw predicted a decrease in allergen-specific proliferation, whereas associations with individual URI symptoms were weaker. The decrease in lymphocyte proliferation as related to the presence of a cold is consistent with the immunosupressive properties ascribed to respiratory viruses, including adenovirus.[43,44] Interestingly, although the proliferative response to allergens was decreased by a cold, there was no significant effect on the response to the mitogen PHA. One possibility is that viral infection inhibits antigen processing and presentation, which can occur in multiple discrete steps preceding T-lymphocyte recognition.[43] In contrast, with the mitogen PHA, the inhibition of antigen processing or presentation would not affect T-lymphocyte responses.

Despite the suppressive influence of a cold on allergen-specific proliferation, the cold-adjusted model demonstrates that increasing levels of Bla g 1 or 2 in the kitchen and home maximum predicted an increase in Bla g 2–specific lymphocyte proliferation (P = .011, .049, respectively). Thus, even in the context of potential immunologic suppression from a viral infection, Bla g 2 lymphocyte proliferation remained a marker of the child's encounter with aeroallergen.

Previous studies have shown a correlation between increases of home levels of Bla g 2 and the physiologic outcome of wheeze,[12] and here we demonstrate that home aeroallergen levels influence lymphocyte proliferation. We observed associations between repeated wheeze in the first 2 years of life and increased allergen-specific and mitogen lymphocyte proliferation (Table II).

In summary, we have examined early childhood immune responses that may effect the development of immunologic responses related to asthma. In a cohort of children at risk for asthma, we analyzed home allergen levels as a measure of exposure as well as lymphocyte responses to the same home allergens. Higher home levels of Bla g 1 or 2, and to a lesser extent Der f 1, were associated with an increased lymphocyte proliferative response. For Fel d 1, high levels do not correlate with an increased proliferative response. Explanations for a lack of allergen-specific proliferation in the setting of high allergen exposure include intrinsic allergenic differences or immunologically acquired nonresponsiveness to allergen. Previous analysis with this cohort indicated that high home levels of Bla g 1 or 2 correlate with increased incidence of a physiologic outcome (ie, repeated wheeze in the first year of life).[12] In concordance, we have found an increased incidence of repeated wheeze in the first 2 years of life to be associated with increased allergen-specific lymphocyte proliferative responses. Taken together, our data indicate that early-life aeroallergen exposure to cockroach allergen predicts later lymphocyte responses. How the differences in immune responses influence the later development of asthmatic phenotypes is under investigation.

We thank Drs David Mark, Carolyn Donovan, Jeffrey Drazen, and David Christiani for their critical reading of this manuscript; Marrisa Barr for coordinating the project; Diane Sredl for statistical analysis; Joanne Maldonis for her secretarial assistance; and all the families who have taken time from their busy schedules to contribute to this project.

REFERENCES

1. Vollmer WM, Osborne ML, Buist AS. 20-year trends in the prevalence of asthma and chronic airflow obstruction in an HMO. Am J Respir Crit Care Med 1998;157:1079-84.
2. Holgate ST. Asthma: a dynamic disease of inflammation and repair. Ciba Found Symp 1997;206:5-34, 106-10.
3. Croner S, Kjellman NI. Natural history of bronchial asthma in childhood: a prospective study from birth up to 12-14 years of age. Allergy 1992;47(Suppl):150-7.
4. Yunginger JW, Reed CE, O'Connell EJ, Melton LJ III, O'Fallon WM, Silverstein MD. A community-based study of the epidemiology of asthma: incidence rates, 1964-1983. Am Rev Respir Dis 1992;146:888-94.
5. Donovan CE, Finn PW. Immune mechanisms of childhood asthma. Thorax 1999;54:938-46.
6. Ownby DR. Environmental factors versus genetic determinants of childhood inhalant allergies. J Allergy Clin Immunol 1990;86:279-87.
7. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life: the Group Health Medical Associates. N Engl J Med 1995;332:133-8.
8. Bergmann R, Woodcock A. Whole population or high-risk group? Childhood asthma. Eur Respir J 1998;27(Suppl):9-12s.
9. Weiss ST. Environmental risk factors in childhood asthma. Clin Exp Allergy 1998;28(5 Suppl):29-34, 50-1.
10. van der Heide S, Dubois AE, Kauffman HF, de Monchy JG. Allergy to mites: relation to lung function and airway hyperresponsiveness. Allergy 1998;53(48 Suppl):104-7.
11. Custovic A, Simpson B, Simpson A, Hallam C, Craven M, Woodcock A. Relationship between mite, cat, and dog allergens in reservoir dust and ambient air. Allergy 1999;54:612-6.
12. Platts-Mills TA, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor allergens and asthma: report of the Third International Workshop. J Allergy Clin Immunol 1997;100(Suppl):S2-24.
13. Korsgaard J. Mite asthma and residency: a case-control study on the impact of exposure to house-dust mites in dwellings. Am Rev Respir Dis 1983;128:231-5.
14. Sporik R, Ingram JM, Price W, Sussman JH, Honsinger RW, Platts-Mills TA. Association of asthma with serum IgE and skin test reactivity to allergens among children living at high altitude: tickling the dragon's breath. Am J Respir Crit Care Med 1995;151:1388-92.
15. Bjorksten B, Holt BJ, Baron-Hay MJ, Munir AK, Holt PG. Low-level exposure to house dust mites stimulates T-cell responses during early childhood independent of atopy. Clin Exp Allergy 1996;26:775-9.
16. Gold DR, Burge HA, Carey V, Milton DK, Platts-Mills T, Weiss ST. Predictors of repeated wheeze in the first year of life: the relative roles of cockroach, birth weight, acute lower respiratory illness, and maternal smoking. Am J Respir Crit Care Med 1999;160:227-36.
17. Litonjua AA, Carey VJ, Burge HA, Weiss ST, Gold DR. Parental history and the risk for childhood asthma: does mother confer more risk than father? Am J Respir Crit Care Med 1998;158:176-81.
18. Chew GL, Burge HA, Dockery DW, Muilenberg ML, Weiss ST, Gold DR. Limitations of a home characteristics questionnaire as a predictor of indoor allergen levels. Am J Respir Crit Care Med 1998;157:1536-41.
19. Chapman MD, Aalberse RC, Brown MJ, Platts-Mills TA. Monoclonal antibodies to the major feline allergen Fel d I, II: single step affinity purification of Fel d I, N-terminal sequence analysis, and development of a sensitive two-site immunoassay to assess Fel d I exposure. J Immunol 1988;140:812-8.
20. Luczynska CM, Arruda LK, Platts-Mills TA, Miller JD, Lopez M, Chapman MD. A two-site monoclonal antibody ELISA for the quantification of the major Dermatophagoides spp allergens, Der p I and Der f I. J Immunol Methods 1989;118:227-35.
21. Milton DK, Johnson DK, Park JH. Environmental endotoxin measurement: interference and sources of variation in the Limulus assay of house dust. Am Ind Hyg Assoc J 1997;58:861-7.

22. Milton DK, Feldman HA, Neuberg DS, Bruckner RJ, Greaves IA. Environmental endotoxin measurement: the kinetic Limulus assay with resistant-parallel-line estimation. Environ Res 1992;57:212-30.
23. Lara-Marquez ML, Deykin A, Krinzman S, Listman J, Israel E, He H, et al. Analysis of T-cell activation after bronchial allergen challenge in patients with atopic asthma. J Allergy Clin Immunol 1998;101:699-708.
24. Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Engl J Med 1997;336:1356-63.
25. Kang B, Chang JL. Allergenic impact of inhaled arthropod material. Clin Rev Allergy 1985;3:363-75.
26. Gold DR, Tager IB, Weiss ST, Tosteson TD, Speizer FE. Acute lower respiratory illness in childhood as a predictor of lung function and chronic respiratory symptoms. Am Rev Respir Dis 1989;140:877-84.
27. Zeiger RS. Atopy in infancy and early childhood: natural history and role of skin testing. J Allergy Clin Immunol 1985;75:633-9.
28. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. Lancet 1999;353:196-200.
29. Prescott SL, Macaubes C, Yabuhara A, Venaille TJ, Holt BJ, Habre W, et al. Developing patterns of T cell memory to environmental allergens in the first two years of life. Int Arch Allergy Immunol 1997;113:75-9.
30. Yabuhara A, Macaubas C, Prescott SL, Venaille TJ, Holt BJ, Habre W, et al. TH2-polarized immunological memory to inhalant allergens in atopics is established during infancy and early childhood. Clin Exp Allergy 1997;27:1261-9.
31. Rowntree S, Cogswell JJ, Platts-Mills TA, Mitchell EB. Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. Arch Dis Child 1985;60:727-35.
32. Rowntree S, Platts-Mills TA, Cogswell JJ, Mitchell EB. A subclass IgG4-specific antigen-binding radioimmunoassay (RIA): comparison between IgG and IgG4 antibodies to food and inhaled antigens in adult atopic dermatitis after desensitization treatment and during development of antibody responses in children. J Allergy Clin Immunol 1987;80:622-30.
33. Holt PG, Britten D, Sedgwick JD. Suppression of IgE responses by antigen inhalation: studies on the role of genetic and environmental factors. Immunology 1987;60:97-102.
34. Mowat AM, Lamont AG, Strobel S, Mackenzie S. The role of antigen processing and suppressor T cells in immune responses to dietary proteins in mice. Adv Exp Med Biol 987;216A:709-20.
35. Holt PG, Macaubas C. Development of long-term tolerance versus sensitisation to environmental allergens during the perinatal period. Curr Opin Immunol 1997;9:782-7.
36. Platts-Mills TA, Chapman MD. Dust mites: immunology, allergic disease, and environmental control. J Allergy Clin Immunol 1987;80:755-75.
37. Platts-Mills TA, Sporik RB, Chapman MD, Heymann PW. The role of domestic allergens. Ciba Found Symp 1997;206:173-89.
38. Arlian LG, Bernstein D, Bernstein IL, Friedman S, Grant A, Lieberman P, et al. Prevalence of dust mites in the homes of people with asthma living in eight different geographic areas of the United States. J Allergy Clin Immunol 1992;90:292-300.
39. Chan-Yeung M, Becker A, Lam J, Dimich-Ward H, Ferguson A, Warren P, et al. House dust mite allergen levels in two cities in Canada: effects of season, humidity, city and home characteristics. Clin Exp Allergy 1995;25:240-6.
40. Chan-Yeung M, McClean PA, Sandell PR, Slutsky AS, Zamel N. Sensitization to cat without direct exposure to cats. Clin Exp Allergy 1999;29:762-5.
41. Ichikawa K, Iwasaki E, Baba M, Chapman MD. High prevalence of sensitization to cat allergen among Japanese children with asthma, living without cats. Clin Exp Allergy 1999;29:754-61.
42. Parvaneh S, Kronqvist M, Johansson E, van Hage-Hamsten M. Exposure to an abundance of cat (Fel d 1) and dog (Can f 1) allergens in Swedish farming households. Allergy 1999;54:229-34.
43. Ploegh HL. Viral strategies of immune evasion. Science 1998;280:248-53.
44. Schrier RD, Rice GP, Oldstone MB. Suppression of natural killer cell activity and T cell proliferation by fresh isolates of human cytomegalovirus. J Infect Dis 1986;153:1084-91.